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LESIONS OF THE LEFT AURICLE IN RHEUMATIC FEVER *

LOUIS GROSS, M.D.

(From the Laboratories of the Mount Sinai Hospital, New York City)

The influence of the nature and topography of tissues on the character of the resultant inflammatory process is better exemplified by rheumatic fever than by most other chronic diseases. Thus, for example, characteristic individual differences are found in the rheumatic lesions of the myocardium, valve rings, valve substance, pericardium, subcutaneous tissues, tendinous insertions and blood vessels, even though some of these may possess structural or cellular peculiarities in common. This relation between inflammatory process and tissue structure is strikingly shown in the left auricular endocardial lesions.

Rather vague and sketchy references to left auricular lesions were made by Corvisart,¹ Burns,² Baillie,³ Wells,⁴ Laennec,⁵ Hope⁶ and Bouillaud.⁷ From these it is often difficult to determine whether the underlying cause was rheumatic fever, bacterial endocarditis or auricular thrombosis.

In 1898 Claude and Levaditi⁸ gave a gross and microscopic description of what appears to have been an old rheumatic, left auricular endocardial lesion with calcification. They noted distended capillaries, inflammatory cells and obliterative vascular changes near the ulcerated (calcific ?) surface. Bacteriological cultures were negative. The case described by Cheadle and Lees⁹ in the same year was undoubtedly one of bacterial endocarditis. This suggestion was made by Poynton, who performed the autopsy.

Huchard's¹⁰ monograph (1903) contains two excellent drawings of

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typical rheumatic auricular lesions illustrating some of the gross and microscopic features of this process. The text description, however, is very vague. In 1914 Harper ¹¹ reported 9 cases of rheumatic infection in childhood. In 1 case the "endocardium of the left auricle was found to be thickened and wavy. This was considered evidence of a former endocarditis." In 1920 Hertel ¹² reported 10 cases of "parietal endocarditis." One of these was a "recurrent endocarditis of the mitral valve." Between the anterior and posterior cusps she observed large deposits, partly warty, partly polypoid, hanging in the heart cavity. These vegetations extended up the left auricular endocardium. An excellent histological description is given of these lesions which Hertel believed were a contiguity process from the mitral valve. However, although the description of this microscopic auricular lesion closely resembles in certain respects the true rheumatic process, the character of the gross lesions and some of the microscopic features suggest the possibility of at least a superimposed bacterial process, in spite of the reported negative bacteriological findings.

The study of this very interesting lesion took on new significance after the publication of the reports by MacCallum (1924-1925).¹³ It is to the great credit of this author that he clearly recognized the essentially rheumatic nature of the left auricular process, gave an excellent description of its gross and microscopic features and, because of his extensive experience in the pathology of rheumatic fever, presented his findings on material which can hardly be subject to question. The most important points brought out by MacCallum were the infiltration of the endocardium and subendocardium with inflammatory cells, the banded appearance of the Aschoff bodies in the endocardium, the presence of necrotic bands of collagen, the edema, the distortion of the elastic lamellae and the extension of the process through the auricular myocardium to the pericardial mantle.

Soon after the publication of the first of these papers, Stewart and Branch ¹⁴ reported a case of rheumatic auricular endocarditis with calcification. The endocardium of the left auricle showed fibrous thickening with irregularity of the surface. The presence of adhesive pericarditis and myocardial Aschoff bodies, together with the clinical history, led the authors to conclude that the auricular lesion was of a rheumatic nature.

In 1926 VonGlahn¹⁶ added materially to our knowledge of this lesion by a description of the auricular changes found in 9 out of 31 cases of rheumatic valvular disease. While no attempt was made to segregate the cases with respect to acuity or chronicity of the rheumatic process in the entire heart, a very careful and detailed description was given of the gross lesions and microscopic findings. In this paper VonGlahn was able to confirm MacCallum's findings and noted in addition the presence of large and small mononuclear cells, as well as polymorphonuclear leukocytes, oriented at right angles to the endocardial wall. He observed fibrin on the surface of some of these lesions and, at times, verrucous deposits. Considerable quantities of fibrin were present in the subendocardium. Capillaries were seen penetrating the endocardial lesion but never beyond the middle portion. Healing took place by means of cells resembling fibroblasts which arranged themselves perpendicularly to the surface in the superficial part of the endocardium, especially between the main connective tissue layer and the endothelium. Later on, elastic fringes and often calcium deposits were seen in these healing and healed areas. Further healing took place by the disappearance of the cellular accumulations.

These descriptions were subsequently amplified by Pappenheimer and VonGlahn,¹⁶ and by VonGlahn and Pappenheimer.¹⁷ In the latter report reference is made to the finding of 3 cases with lesions in the right auricle. Since the publication of these papers several new cases have been described by Shaw,¹⁸ Perla and Deutch,¹⁹ and by Klinge,²⁰ largely confirming the previously reported observations.

On reviewing the findings reported by the above mentioned authors, it appeared desirable to investigate these lesions on a much larger series of material, laying especial emphasis on the correlation between the gross and histological findings in the left auricle and the clinical course of the disease. This report will concern itself with a study of 87 rheumatic cases. Sixty-seven of these were active and showed Aschoff bodies in the myocardium, and 20 showed chronic valvular disease of the rheumatic variety but without evidence of activity either clinically or pathologically and with no demonstrable Aschoff bodies in the myocardium. The grouping as to activity and inactivity was based on the criteria outlined by Rothschild, Kugel and Gross.²¹ Particular care was taken to avoid material which in any way indicated the possibility of a coexisting bacterial endocar-

ditis. A careful study of the clinical records and pathological specimens made it possible to divide the material into the following groups:

GROUP 1. Active cases where death took place in the first attack or where one preceding attack occurred within 1 year of the fatal outcome.

GROUP 2. Active cases in which one previous attack occurred at least 2 years previous to the fatal outcome.

GROUP 3. Active cases with repeated attacks, death occurring during an acute recurrence.

GROUP 4. Active cases where death was caused by decompensation without clinical evidence of a final recurrence. Some of these cases had no previous history of rheumatic fever.

GROUP 5. Inactive cases of chronic valvular disease of the typical rheumatic variety.

The technical methods used were essentially the same as those previously described by Gross and Ehrlich.²² The findings here reported are based on a study of the routine left auricle sections (L.A.) and the auricular portions of the routine mitral valve posterior sections (M.V.P.) obtained in the standardized procedure suggested by Gross, Antopol and Sacks.²³

Before describing the findings in this material it is advisable to present very briefly the histological characteristics of the normal left auricular endocardium, with special reference to the age period changes. It will be found that among the changes produced by rheumatic fever in the left auricular endocardium there occur alterations in the relations of the connective tissue, smooth muscle and elastic tissue components. Since alterations also occur as the result of age, it is necessary to have a clear-cut conception of these processes in order to differentiate the changes that are of normal evolutionary origin from those that may be due to the rheumatic process. This is particularly true for the findings in the inactive cases.

Descriptions of the histology of the left auricular endocardium have been reported by Königer,²⁴ Nagayo,²⁵ Miller and Perkins,²⁶ and by Perkins and Miller.²⁷ The descriptions by Königer and Nagayo appear to be unnecessarily complex and too rigid to fit the not inconsiderable variations found in the normal, human, left auricular endocardium. In the reports by Miller and Perkins, and Perkins and Miller, the age period changes in the left auricular endocardium

are described in a 2 day old infant, a 35 year old individual and an 85 year old individual.

In order to obtain a more plastic impression of the age period changes in a fairly representative number of cases, 50 normal hearts were chosen for study. These specimens were carefully selected to rule out such conditions as may be expected to affect the cardiovascular system. An examination of this material reveals the following histological and topographical features of the left auricular endocardium and their changes from birth to the eighth decade of life.

HISTOLOGY AND AGE PERIOD CHANGES OF THE LEFT AURICULAR ENDOCARDIUM

The fibro-musculo-elastic membrane lying internally to the left auricular myocardium may be divided into two layers, *i.e.* the endocardium proper and the subendocardium (Fig. 1). The former consists at birth of somewhat closely packed, anastomosing delicate sheets of elastic tissue separated by collagen fibrils. The elastic membranes lie parallel to one another and to the lumen of the auricle, running for the most part transversely to the axis of the blood stream flow. Dividing this endocardial layer arbitrarily into three equal zones, *viz.* inner third, middle third and outer third, it may be said that there are no conspicuous concentrations of the elastic tissue in any of the zones but that the outermost one occasionally contains a few smooth muscle fibers with their axis generally parallel to the elastic lamellae. Apart from these, the endocardium shows a large number of fibroblasts with fairly abundant cytoplasm and somewhat vesicular rounded nuclei. The cells are embedded in a mucinoid, more or less basophilic matrix. Within a few months after birth the cytoplasm disappears and the nuclei become denser and somewhat elongated. Scattered, large mononuclear cells, occasional lymphocytes and rare polymorphonuclear leukocytes may also be seen. These cells occur rather infrequently until about the fourth decade of life when they practically disappear from the normal endocardium. The number of fibroblastic nuclei appears to fall off sharply within the first year of life. They generally occupy the middle and outer thirds of the endocardium. From the third decade on they become very sparse. No blood vessels are seen in the normal endocardium.

The endocardium is covered by flat endothelial cells. Between these and the outermost elastic lamellae there are found a few scattered cells apparently derived from the primitive mesenchymal layer which, because of their multipotential properties under certain inflammatory conditions, will be referred to as the "mesenchymal layer." It is to be noted that this layer is most inconspicuous in the normal left auricle.

Immediately external to the endocardium proper, and intimately bound up to it, lies the subendocardial layer. This consists of larger and denser collagenous masses separated by interlacing elastic fibers which are thicker than those of the endocardium proper and generally run at right angles to the latter. The outermost zone of this subendocardial layer may at times take on a looser structure and merge with the collagenous framework of the adjacent auricular myocardium. A few scattered capillaries and very rare mononuclear cells are normally present in the subendocardium. Lymphocytes and polymorphonuclear leukocytes are practically never seen. The capillaries are often more conspicuous when fat tissue is present.

During the second year of life the regularity of the endocardial pattern may be somewhat disturbed. The elastic fibers may show focal concentrations or, in places, be missing. Thus, the beginnings of a mosaic pattern are already noted. This becomes more frequent during the second half of the first decade. About the end of the first decade an occasional specimen shows a somewhat greater concentration of smooth muscle fibers in the outermost endocardial zone. These run occasionally obliquely, but for the most part, transversely. At times the innermost endocardial layers may be scant in elastic fibers and present an appearance somewhat resembling the inflammatory structure which will be referred to as an "endocardial reduplication." These are, however, generally easily distinguishable from true reduplications during the first four decades of life, after which they occur with considerably greater frequency and can seldom be distinguished from the type of reduplication often found in chronic valvular disease of long duration.

From the second decade on, occasional specimens show a less distinct differentiation between the endocardium and subendocardium. During the third decade the subendocardium begins to show fat accumulations. As indicated before, these frequently contain a larger number of capillaries. The smooth muscle fibers, which gen-

erally occupy the outer zone of the endocardium proper and occur in more or less compact masses, become decidedly more conspicuous in the fourth decade and may produce irregularities in the distribution of the elastic lamellae, with the formation of elastic-muscular mosaics.

In the sixth decade of life a new pattern appears. The superficial layers of the elastic tissue may be either concentrated or quite defective. The inner zone of the endocardium generally shows atrophy of the elastic fibers with, at times, disappearance. The middle zone often shows dense concentrations of elastic fibers. The outer zone frequently contains conspicuous smooth muscle bundles, at times intermingled with dense elastic concentrations. The subendocardium frequently contains considerable fat tissue.

In the seventh decade the elastic fibers become extremely scant, being concentrated in irregular collections; the smooth muscle components are quite conspicuous and endocardial reduplications internal to the innermost elastic lamellae are frequently encountered. These reduplications are generally narrow, not elastified, of more or less even thickness and rest on a fairly heavy, elastic limiting membrane. The eighth decade frequently shows distinct atrophy of the endocardium with, generally, hypertrophy of the smooth muscle elements.

MACROSCOPIC APPEARANCE OF RHEUMATIC AURICULAR ENDOCARDITIS

The macroscopic appearance of the rheumatic auricular lesions has been described in detail by VonGlahn.¹⁵ The chief points brought out by this author are the predilection site of the posterior wall of the left auricle, at times spreading over almost the entire endocardial surface into the auricular appendage and up to the orifices of the pulmonary veins; the rare involvement of the foramen ovale; the appearance of irregular low ridges and hillocks, separated by furrows with no definite pattern; the smooth, glistening, at times slightly dull, irregular surface with, rarely, distinct projections resembling vegetations; the tawny gray color in the more acute lesions and grayer and translucent appearance of the older ones; and in the more extensive process, the flat or delicately ridged plaques of yellow color.

To the above résumé may be added the fact that not infrequently the only macroscopic evidences of endocardial lesions are the presence of very inconspicuous, flat, often rounded plateaus, sometimes measuring no more than 2 mm. in diameter, generally delicate pink but, at times, no different in color from the remainder of the left auricular endocardium. These delicate elevations merge imperceptibly with the adjacent tissues and are best brought out by sponging the inner surface of the auricle and allowing the light to fall on the lesion in such a way as to emphasize the shadows, much as is done in the photography of flat projections.

It is to be noted that the normal endocardium often displays a cross weaving of elastic fibers in somewhat sharply cut geometric pattern. These may appear as slightly raised, white or gray ridges which, however, are practically always in the form of straight lines. The flat rheumatic auricular lesions differ from these geometric elastic patterns of the normal auricular endocardium in their rounded contour, greater irregularity, broader base and, sometimes, in their pinkish color.

The only statistics available on the incidence of macroscopic left auricular rheumatic lesions are those by VonGlahn, who observed 9 instances among 31 cases (29 per cent), and Thayer,²⁸ who noted acute or chronic mural endocarditis of the left auricle in 10 out of 25 cases (40 per cent). Gross auricular lesions were observed in 80 per cent of the series of rheumatic hearts which form the basis of this report. The highest incidence was in Group 3 of the above mentioned clinical classification (repeated attacks) where they were found in every instance. The lowest incidence was in Group 5 (chronic valvular disease without Aschoff bodies) where they were found in approximately half the cases. Macroscopic lesions in the right auricle were very mild, not nearly as easily discernible as those of the left auricle and relatively infrequent. Their histological characteristics are similar to those of the left auricular lesions.

MICROSCOPIC APPEARANCE OF RHEUMATIC AURICULAR LESIONS

As has been indicated by previous authors, the auricular lesions in rheumatic fever may present a variety of pictures depending on the acuity of the process and on the individual reactions of the tissues. These reactions in turn may possibly be influenced by the immuno-

logical state of the individual. An analysis of these histological changes indicates that they may be roughly classified into five types, each corresponding to one of the clinical groups mentioned above.

Group 1

Histology of the Left Auricular Lesion Found in Active Cases Where the Individual Died Either in the First Attack or Where One Preceding Attack Occurred Within 1 Year of the Fatal Outcome

There were 17 cases in this group, ranging in age from 17 months to 19 years. The endocardium proper in every specimen showed varying grades of infiltration with inflammatory cells (Fig. 2). These were generally polymorphonuclear leukocytes and lymphocytes, as well as occasional eosinophiles, plasma cells and large mononuclear cells. Many of the cells were oriented at right angles to the auricular lumen, appearing often as ameboid streamers. In approximately half the cases the infiltration was very marked. In almost every specimen there were noted palisades or banded arrangements of these cells on either side of swollen elastic and collagen fibers. There did not appear to be any site of predilection within the auricular endocardium for these palisades. Apart from these, however, the infiltration was generally diffuse with numerous, focal, intense accumulations scattered irregularly throughout the several zones of the endocardium. In approximately half the cases there were present stratified cellular accumulations with histological properties similar to the cellular components entering into the formation of Aschoff bodies. Thus, the cells possessed owl-eyed, fibrocytoid and pyknotic nuclei and were surrounded by rather abundant basophilic cytoplasm with ragged edges. These cells also appeared to be limited by the adjacent elastic and collagenous bands (Fig. 3) and sometimes arranged themselves around fragments or sheets of swollen eosinophilic collagen. These lesions will be referred to as endocardial Aschoff bodies.

Besides these cellular accumulations the endocardium, as indicated, generally showed conspicuous bands and foci of swollen eosinophilic collagen (fibrinoid change). The elastic fibers almost invariably showed a definite departure from the normal consistence and topography. Instead of the regular lamellated arrangement seen in normal controls, particularly in the younger age periods,

many of the endocardial lesions showed spreading apart of the elastic lamellae (Fig. 4) and deviation from their parallel arrangement so that arches were formed, often directed toward the subendocardium. Occasionally, the membranes showed rupture with irregular patches of thinning; in other areas they were thickened and concentrated (Fig. 5). For simplicity of description such irregularities in the distribution of the elastic membranes will be referred to as "elastica distortions." Approximately one-third of the cases showed marked edema of the endocardium. This generally occurred in the area showing greatest cellular accumulations.

As previously indicated, there is a tendency for the smooth muscle of the endocardium to increase with advancing age. This muscular stratum is generally but not invariably situated in the outermost zone of the endocardium and usually occurs in more or less compact form. In the typical auricular lesion falling in this clinical category, the smooth muscle elements may increase in number (Figs. 6 and 7). They are sometimes irregularly arranged; the bundles may be separated into smaller discrete islands and occupy various zones of the endocardium proper.

It has also been shown that the normal endocardium possesses no discernible capillaries (as opposed to the subendocardium, in which capillaries are invariably seen). In the auricular lesion under description there is seen in the majority of instances a penetration of capillaries from the subendocardium into the endocardium proper. These are frequently seen to lie between the smooth muscle bundles, often arranged at right angles to the endocardial surface (Fig. 7). In approximately half the cases they penetrate to the middle zone of the endocardium (Figs. 8 and 10) and in about one-third of the cases they penetrate as far as the inner zone (Fig. 9). This phenomenon will be referred to as "capillary penetration," although it is to be noted that in a few instances the penetrating vessels are of arteriolar structure.

Perhaps the most important feature of this lesion is the endocardial "reduplication." This is a term intended to designate the formation of a new layer of tissue situated between the innermost elastic lamella and the auricular endothelial lining. These reduplications occur in several different forms. In the clinical group under discussion the most frequent form is the embryonal uncovered type. This consists of a not inconspicuous layer of mucinoid-staining mate-

rial which may attain a width approximately equal to that of the endocardium proper (Fig. 6). Within the matrix of this material there are seen stellate and spindle cells often running at right angles to the elastic membranes and consisting of vesicular nuclei surrounded by a rather hazy, faintly staining cytoplasm. Inasmuch as these cells apparently possess the multipotentiality of mesenchymal tissue (transforming themselves usually into fibroblasts, smooth muscle and, rarely, bone) they are referred to here as embryonal mesenchymal cells. Occasionally in this group an elastic membrane is seen covering this reduplication (covered embryonal reduplication). It is apparently this lesion that VonGlahn considered one of the healing processes of the endocardial lesion.

In this clinical group more than half the cases show these reduplications as single strata. Occasionally, however, two strata are seen separated from one another by an elastic membrane. These are referred to as multiple reduplications (Figs. 6, 10 and 12). Another form of reduplication is the edematous type. This was seen only once and consisted of a rather gelatinous tissue infiltrated with small, round, inflammatory cells (Fig. 4). Although occurring infrequently in this group, the covered reduplication presents another variant. This consists of an endocardial reduplication, often with an embryonal matrix, lying inside the innermost auricular endocardial elastic membrane and showing various grades of elastification with more or less parallel rows of elastic lamellae (elastified reduplication) (Fig. 8). Another form of reduplication is the dense collagenous variety. As its name indicates, this consists of rather dense collagen bundles (Figs. 9, 10 and 11) which may or may not be penetrated with a varying amount of elastic tissue. This occurred only once in this series. Smooth muscle cells in the endocardial reduplications were seen only once in this group (Fig. 10).

In this clinical group the subendocardium invariably shows evidence of inflammatory change (Figs. 2, 3 and 4). This generally consists of a marked infiltration between the collagenous bundles. The latter may or may not show eosinophilic swelling. The cellular components are similar to those mentioned as forming the characteristic infiltration of the endocardium. The infiltration is generally diffuse but may be focal. Both the inflammatory cells as well as edema produce, at times, a marked increase in the width of the subendocardium (Figs. 3 and 4). In approximately one-third of the cases Aschoff

bodies were present in the subendocardium, these being of a somewhat modified mosaic pattern with the characteristic cells lying in the crypts between the collagenous bundles. The elastic fibers of the subendocardium also show considerable modification, such as thickening, swelling, rupture, concentration and, at times, disappearance.

The capillary component of the subendocardium generally shows a very definite increase in their number. Not infrequently they are larger, more conspicuous, often oriented toward the auricular lumen, sometimes with endothelial buds and swollen endothelial cells. One case in this group showed the characteristic, intimal, musculo-elastic hyperplastic changes similar to those described by Gross, Kugel and Epstein²⁹ as occurring in other coronary vessels in the heart in rheumatic fever (Fig. 11). Not infrequently delicate vascular channels are seen to be distended with lymphocytes (Fig. 1). This was pointed out by MacCallum.

Approximately half the cases showed considerable hypertrophy of the myocardium (Fig. 12). As Sacks³⁰ has indicated, such hypertrophy is not necessarily associated with valvular defects but appears to be a direct stimulating effect of the exciting agent. In the interstitium between the myocardial bundles several cases showed edema and early fibrosis. Practically every specimen showed some form of cellular infiltration, often with lymphocytes, sometimes with polymorphonuclear leukocytes, occasionally plasma cells, eosinophiles and large mononuclear cells (Fig. 1). These were arranged either diffusely or focally and very often showed a contiguity with the subendocardial infiltration on the one hand and with an inflammation of the pericardium on the other. Apart from these non-specific cellular infiltrations, more than one-third of the specimens showed myocardial Aschoff bodies of various types.

As indicated in a previous report,²⁹ the vascular lesions occurring in the left auricular wall in acute rheumatic fever are not conspicuous or characteristic. Nevertheless, the majority of cases showed vessels with glassy medial hypertrophy, and with medial hypertrophy.* In some cases the capillaries were rather conspicuous. Rarely giant medial hypertrophy with metallaxis was encountered. In 2 cases intimal fibrosis was noted.

In every specimen some form of pericardial inflammation was

* For a description of these lesions see Gross, Kugel and Epstein.²⁹

noted. In more than half the cases this consisted of a microscopic, mild or marked infiltration, generally with lymphocytes, occasionally with polymorphonuclear leukocytes, eosinophiles, plasma cells and large mononuclear cells. These infiltrations tended to occupy the outermost zones of the pericardium. In 2 cases pericardial Aschoff bodies were seen. Rarely, eosinophilic swelling of the collagen was noted. The pericardial capillaries were generally quite conspicuous. In 2 cases the vessels showed small thrombi, in 1 case there was seen polypoid endarteritis, giant medial hypertrophy with metallaxis and intimal musculo-elastic hypertrophy.

In one-third of the cases a distinct fibrinous pericarditis was present. It is to be noted, however, that this figure represents the incidence of this lesion as it was observed in the left auricular section studied. Macroscopic pericarditis, either fibrinous or adhesive, was observed on some portion of the entire heart in 80 per cent of the cases in this clinical group.

Group 2

Histology of the Left Auricular Lesion Found in Active Cases Where One Previous Attack Occurred at Least 2 Years Previous to the Fatal Outcome

There were 10 cases in this group, ranging in age from 7 to 34 years. The average endocardial lesion resembled very closely that described for the first group. For the sake of simplicity its chief histological features are listed seriatim as follows:

1. Palisades were observed in only 4 of the 10 cases, as compared to 15 of the 17 cases in Group 1. These palisades did not differ essentially in their histological characteristics from those previously described. The same may be said for the incidence of eosinophilic swelling of the collagenous tissue, which closely paralleled the incidence and extent of the cellular infiltration.
2. Endocardial Aschoff bodies were found of the same type as those previously described. The incidence, however, was appreciably lower (2 out of 10 cases).
3. Inflammatory cell infiltrations, elastica lesions, capillary penetration and increase in the smooth muscle component of the endocardium were similar to that described in Group 1.

4. The most conspicuous difference from the first group lies in the nature and incidence of the reduplications. These were found in 7 out of the 10 cases. Multiple reduplications were seen in 1 case. All the reduplications were elastified. Some were covered. Two were of the dense collagenous variety and one showed a smooth muscle component.
5. As in Group 1, the width of the subendocardium was increased in approximately half the cases (Fig. 9).
6. Infiltration of the subendocardium with inflammatory cells occurred with about the same frequency as in Group 1, with perhaps a lower grade of intensity in approximately half the cases.
7. Subendocardial Aschoff bodies were found in only 1 case of the 10, as compared to 5 of the 17 in Group 1.
8. The increase in subendocardial vascularization was approximately the same as in Group 1, as was the nature of the vessels. One case showed arterioles and arteries with hypertrophied walls.
9. Eight out of the 10 cases showed myocardial hypertrophy. In the majority of these the hypertrophy was marked.
10. The interstitial myocardial infiltration, which was on the whole somewhat less marked than that in Group 1, occurred in 7 out of the 10 cases. Aschoff bodies were found in 4 cases.
11. Myocardial fibrosis was of about the same extent and incidence as in Group 1.
12. The myocardial vascular lesions were somewhat more varied. Thus, in 2 cases some of the blood vessels showed proliferation and desquamation of the endothelium; 1 case showed giant medial hypertrophy with metallaxis; 2, intimal fibrosis; 2, plugging of vessels with small thrombi; 1, intimal musculo-elastic hyperplastic changes, and 1, intimal elastification even in an early age period.
13. The incidence and type of microscopic pericarditis is similar to that described in Group 1. In 2 cases section of the left auricle showed adhesive pericarditis; 1 case showed a variety of rheumatic vascular lesions in the pericardium similar to those described in Group 1. (Macroscopic pericarditis, either fibrinous or adhesive, was observed on some portion of the entire heart in 7 of the 10 cases in this clinical group.)

Taken as a whole, the most conspicuous differences between the left auricular lesions in the first group and those in this clinical group lie in the lower incidence in the latter of palisade formations and of Aschoff bodies in the endocardium, subendocardium and myocardium; in the somewhat higher incidence and qualitative differences in the reduplications; and in the milder form of the interstitial myocarditis.

Group 3

Histology of the Left Auricular Lesion Found in Active Cases With Repeated Attacks, Death Occurring During an Acute Recurrence

There were 12 cases in this clinical group, ranging in age from 4½ to 36 years. The average endocardial lesion showed decidedly greater changes from the type described in Group 1 than did the previously described group. The following are the chief histological features:

1. While infiltration of the endocardium, as in the previous groups, was found in every case, this was marked in only 5 cases. The character of the cells and the incidence of eosinophilic swelling of the collagen was about the same. As in the previously described group, palisades were found less frequently than in Group 1. These were noted in 7 cases, in 5 of which the cells were of the large mononuclear variety.
2. Endocardial Aschoff bodies were found in 2 cases.
3. The elastica lesions were similar to those described in Group 1. They were found in every case.
4. Capillary penetration occurred in 11 of the cases. In 4 this was quite marked. In the rest of the cases penetration was very inconspicuous and affected only the outermost musculo-elastic zone.
5. The smooth muscle increase was perhaps more definite in this group.
6. The most important difference from the previous groups lay in the incidence and type of reduplications. Thus, 6 cases of the 12 showed multiple reduplications, of which 3 possessed triple reduplications. Altogether, reduplications were found in 11 cases. The majority of them were elastified. A few of them were of the embryonal type and 2 showed smooth muscle components.

7. In practically every instance the subendocardium was somewhat increased in width. The infiltration, which was qualitatively of the same type as previously described, was marked in 7 cases.
8. In 3 cases subendocardial Aschoff bodies were present.
9. The increase in subendocardial vascularization, though still high in incidence (9 cases), was somewhat lower than previously described.
10. Eleven of the 12 cases showed marked myocardial hypertrophy.
11. Fibrosis between the myocardial bundles was found in 7 of the 12 cases, in 1 of which elastification was also present, even though in a relatively early age period (17 years).
12. In 4 cases inflammatory cell infiltration was marked. In 3 there was no infiltration noted. In the rest the infiltration was mild. Myocardial Aschoff bodies were found in 2 cases.
13. Vascular lesions of the myocardium occurred in even greater variety and frequency in this group. Thus, 3 cases showed intimal elastification; 2, intimal fibrosis; 4, thickening of the myocardial arteries or arterioles; and 1, intimal musculo-elastic hyperplastic changes.
14. Eight cases showed organized or organizing pericarditis, a much higher incidence than was found in the previous groups. Typical rheumatic vascular lesions were found in 1 case. (Macroscopic pericarditis, either fibrinous or adhesive, was observed on some portion of the entire heart in 10 of the 12 cases in this clinical group.)

Considered as a whole, the features of this group are the tendency for the palisade formations to consist of large mononuclear cells, the higher incidence of multiple reduplications and adhesive pericarditis, and the lower incidence of interstitial myocarditis.

*Group 4**Histology of the Left Auricular Lesion Found in Active Cases Where Death was Caused by Decompensation Without Clinical Evidence of a Final Recurrence. Some of these Cases Had No Previous History of Rheumatic Fever*

There were 28 cases in this clinical group, ranging in age from 18 to 62 years. The average auricular lesion showed a decided decrease in active inflammatory phenomena. The following are the chief histological features:

1. Only 5 cases of the 28 in this group showed marked infiltrations of the endocardium; these were qualitatively similar to those described previously. Five cases showed no infiltration. In the remaining cases there was present only a mild grade of generally lymphocytic infiltration. Edema was also less marked and less frequent in this group.
2. Endocardial Aschoff bodies were found in only 4 of the 28 cases.
3. The incidence of palisade formations was decidedly lower, only 3 cases presenting these lesions. These were of the larger mononuclear cell variety. Eosinophilic swelling of the collagen occurred in only 3 cases, in 1 of which it was quite pronounced.
4. The smooth muscle components of the endocardium were very definitely increased, even after making allowance for the fact that the majority of the cases in this group belong to older age periods.
5. The elastica lesions were of the patchy variety and somewhat difficult to distinguish from age period changes (Fig. 11).
6. Nine cases showed multiple reduplications, of which 3 were triple reduplications. Altogether, 27 cases showed reduplications, generally of the elastified or dense collagenous variety. These were usually covered. In 1 case (Fig. 11), the reduplication was of the flat, collagenous, and somewhat elastified variety which, as will be noted later, is very characteristic of the cases belonging to the next clinical group, *i.e.* chronic valvular disease without activity. One

reduplication was of the embryonal type. Three of the reduplications showed smooth muscle components. One case showed calcific deposits on the superficial layers of the endocardium.

7. The width of the subendocardium was increased in the majority of instances.
8. Five of the 28 cases showed marked cellular infiltrations of the subendocardium; 17 cases showed mild infiltrations; 6 cases showed none.
9. Subendocardial Aschoff bodies were found in 7 of the 28 cases.
10. The vascularization of the subendocardium was increased in the majority of the cases. In 1 there was noted arteriolar hypertrophy; intimal musculo-elastic hyperplastic lesions were noted in 2.
11. In practically every case the myocardium was markedly hypertrophied.
12. In 18 cases there was no infiltration of the interstitial tissue of the myocardium. When it occurred it was generally mild and focal, consisting usually of lymphocytes and histiocytes. In 1 case myocardial Aschoff bodies were present.
13. The incidence of myocardial fibrosis was similar to that previously described. In addition, however, elastification occurred in 3 cases (in the somewhat later age periods).
14. The vascular lesions of the myocardium consisted of intimal elastification in about half of the cases. It is to be noted again that these occurred in the somewhat later age periods.
15. The pericardial lesions were on the whole mild. However, fibrinous pericarditis occurred in 1 case. Peculiar infoldings of the pericardial mantle with large swollen lining cells were found in 2 cases. Pericardial Aschoff bodies were found in 1 case. (Macroscopic pericarditis, either fibrinous or adhesive, was observed on some portion of the entire heart in 9 out of the 28 cases in this clinical group.)

Considered as a whole, the features of this group are the lower incidence of endocardial infiltration, palisade formation, Aschoff bodies and myocardial infiltration, and the higher incidence and peculiarity of the reduplications as well as the mildness of the pericardial lesions.

*Group 5**Histology of the Left Auricular Lesion Found in Inactive Cases of Chronic Valvular Disease of the Typical Rheumatic Variety*

There were 20 cases in this clinical group, ranging in age from 11 to 80 years. The average auricular lesion was marked by its extreme indolence. The following are the chief histological features:

1. Infiltrations of the endocardium occurred in practically every specimen. However, these were all very mild, consisting of scattered lymphocytes, occasional amebocytes and often large mononuclear cells. In many instances these infiltrations could not be distinguished from the occasional cellular infiltrations of the normal endocardium. However, inasmuch as the latter showed practically no infiltration after the fourth decade, its occurrence in many of the older cases of this clinical group was of some significance.
2. In 1 case there occurred a palisade formation of large mononuclear cells. This was associated with a moderate amount of eosinophilic swelling of the collagen. Edema was not found.
3. Aschoff bodies were not present in the endocardium.
4. A great variety of elastic changes was found in this group. None of them, however, showed the characteristic separation and stretching of the elastic fibers seen in the more active lesions. They consisted generally of exaggerations of the normally occurring age period changes of the elastic tissue, from which they were difficult and often impossible to distinguish. On the whole, it may be said that the elastic distribution in the endocardium in cases falling into this group is distinctly irregular, patchy, with, not infrequently, areas of cross-weaving of the elastic fibers and accumulation into compact bundles forming a mosaic with areas in which the elastic tissue was extremely sparse.
5. As in the previously described groups, the smooth muscle of the endocardium was increased. This, however, was frequently difficult to judge because of the older age periods in which most of these cases fell.
6. The endocardium itself was lightly infiltrated with calcium salts in 1 case.

7. All the cases showed reduplications. In 2 cases these were multiple. In the great majority of cases the reduplications were of the flat, dense, elastified variety. Smooth muscle was found in one reduplication.
8. Many of the specimens showed a moderate widening of the subendocardium. This consisted frequently of fibrosis, to which there was often added a fat cell component.
9. Infiltration of the subendocardium was generally very mild and consisted of lymphocytes. In the somewhat younger cases the infiltration tended to be slightly more conspicuous. Two cases showed no infiltration. No subendocardial Aschoff bodies were present.
10. About half the cases showed increase in vascularization without distinctive features.
11. All the cases showed a very marked hypertrophy of the auricular myocardium.
12. Infiltration of the myocardial interstitium was even less marked than in the previous group. It occurred in approximately half the cases and was generally mild and focal.
13. Fibrosis of the myocardium occurred in about one-third of the cases.
14. The most conspicuous myocardial vascular lesion was congestion of the capillaries, which occurred in about one-third of the cases. For the rest, the arterioles appeared to be somewhat hypertrophied and intimal elastification was somewhat more frequently found. However, these corresponded to the age period changes to be expected in this group.
15. Pericardial lesions were found in only 12 cases and consisted of mild scattering of lymphocytes. (Macroscopic pericarditis, either fibrinous or adhesive, was observed on some portion of the entire heart in 4 out of the 20 cases in this clinical group.)

Considered as a whole, this group of cases is notable for the extreme mildness of infiltrative phenomena, for the absence of Aschoff bodies and for the possession of the characteristic, flat, elastified reduplications.

DISCUSSION

Although the blocks of tissue on which this study was made represent single specimens from each case cut according to our routine procedure and generally without any special effort to include macroscopic lesions, every specimen showed some variety of histological lesion that can be reasonably attributed to rheumatic fever. It must not be inferred from this, however, that the lesions were always so distinctive as to permit a diagnosis of rheumatic fever solely on the auricular findings. Particularly in Groups 4 and 5 it is not infrequently difficult to distinguish the essential inflammatory lesions of rheumatic origin from the confusing concomitant age period changes. On the other hand, in the clinical groups which represented the more active cases (Groups 1, 2 and 3) the lesions were very varied, generally quite conspicuous and presented a sufficient number of individual pathological processes on which it is possible to make a diagnosis of rheumatic fever. These lesions consist of edema and marked infiltrations of the endocardium with inflammatory cells, the banded appearance of some of these cellular aggregations, the presence of eosinophilic swelling of the collagen, the presence of Aschoff bodies in the endocardium, subendocardium and myocardium, the distortion of the elastic tissue and the widening, hypercapillarization and marked infiltration of the subendocardium. To these, furthermore, may be added the two important features of capillary penetration into the endocardium and the presence of reduplications. A less important feature is the presence of scattered and increased smooth muscle elements in the endocardium, together with smooth muscle in the reduplications proper. In none of the material examined was there present a necrosis of the superficial layers of the endocardium of sufficient intensity to warrant the term "verrucous change." Together with these endocardial and subendocardial lesions these first three groups also presented a very high incidence of myocardial hypertrophy and interstitial inflammatory cell infiltration. In approximately half the cases microscopic section of the left auricle showed some pericardial lesion.

In the more chronic clinical groups (4 and 5) the inflammatory phenomena of the endocardium and subendocardium were present in almost every instance, but very much milder. Aschoff bodies occurred in extremely low incidence or not at all (Group 5), and the

subendocardial inflammatory phenomena were also less frequent and milder, as were those in the myocardium and pericardium. On the other hand, the almost invariable presence of reduplications, sometimes multiple, the penetration of capillaries into the endocardium, when present, the marked hypertrophy of the myocardium and the presence in the majority of cases of some form of pericardial lesion, together with the increase and irregularity of the endocardial smooth muscle, readily constituted criteria on which to entertain at least a suspicion of rheumatic fever.

Of great interest is a consideration of the differences in the microscopic lesions between each of the five clinical groups. Certain phenomena are observed throughout all the groups, even though usually in different proportions. On the other hand, each group presents certain characteristics of its own which are both of a quantitative as well as of a qualitative value. Thus, active inflammation of the endocardium and subendocardium, as well as of the myocardium, is generally noted in Groups 1, 2 and 3. These inflammations are less marked in Group 4 and extremely mild in Group 5. This is true for the cellular infiltrations, edema and eosinophilic collagen swelling. The incidence of small round cell inflammatory infiltration shows a rather abrupt decline in Groups 4 and 5 where large mononuclear cells are more frequently found. As previously noted, palisades were observed with considerable frequency in Groups 1, 2 and 3. They were infrequent in Group 4 and occurred only once in Group 5. The same may be said of the incidence of Aschoff bodies which was high in only the first group. Capillary penetration of the endocardium is seen in all the groups, being most marked, however, in the first four. As noted, inflammatory infiltrations of the subendocardium occur in all the groups, but are most marked in the first three. Increased vascularization of the subendocardium is found in all the groups, chiefly in Groups 2, 3 and 4. The incidence and type of pericarditis varies somewhat. It is somewhat similar in the first two groups, where it is generally of the lymphocytic or fibrinous variety. Organizing pericarditis, however, appears conspicuously in Group 3. The total incidence falls in Group 4 and especially in Group 5.

Besides the conspicuous difference in the incidence of Aschoff bodies, cellular infiltration, palisade formation and eosinophilic swelling of collagen, the incidence and types of endocardial redupli-

cations constitute the most characteristic difference between each of the five groups. Thus, in Group 1, 10 of the 17 cases showed reduplications usually single and of the embryonal, uncovered variety. In Group 2 the incidence is higher (7 out of 10), the reduplications are generally single but the type is changed to either the elastified or covered variety. In Group 3 multiple reduplications begin to make their appearance. The majority of lesions are elastified. Some are triple. In Group 4 the reduplications are multiple in approximately one-third of the cases; in the majority they are elastified or dense and covered. In Group 5 the great majority of the reduplications are of the flat, dense, elastified variety. It is seen, therefore, that the appearance of these reduplications constitutes one of the most significant differences in the lesions peculiar to the several clinical groups of rheumatic fever.

In spite of the different histological features of the several clinical groups of rheumatic fever pointed out in this report, it must be borne in mind that the observations were made on too limited a number of cases on which to place complete reliance on the statistics submitted, nor indeed is it considered justifiable as yet to attempt such fine distinctions in the appraisal of a given case. The observations are presented as a suggestive indication for further study and with the thought that other associated lesions in the heart and elsewhere in the body as a whole may reflect, at least to a certain extent, the differences in the reactions of the tissues to rheumatic fever as determined by the clinical course of the disease.

SUMMARY AND CONCLUSIONS

Gross and histological observations on the left auricle, based on an examination of 87 rheumatic hearts, are described. The material is classified into five clinical groups, depending on the course of the disease. It is shown that macroscopic lesions of the left auricle occur in 80 per cent of the cases and microscopic lesions in 100 per cent. In the acute cases the lesions are very significant and characteristic. In the chronic cases they are considerably milder and often difficult to differentiate from normally occurring histological changes. A description is also given of the age period changes of the normal left auricle, as observed in 50 hearts.

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DESCRIPTION OF PLATES

PLATE 100

FIG. 1. Normal left auricle. Age 3 months. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

Arrow indicates division between A, endocardium and B, subendocardium; C = myocardium.

FIG. 2. Left auricle from active case of rheumatic fever. Age 18 years. Low power. Hematoxylin and eosin stain.

A = edematous portion of endocardium; B = infiltration of endocardium with inflammatory cells in banded arrangement; C = markedly infiltrated subendocardium. Note hypercapillarization. D = marked infiltration of myocardial interstitium.

FIG. 3. Left auricle from active case of rheumatic fever (injected specimen). Age 12 years. Low power. Hematoxylin and eosin stain.

A = endocardium infiltrated with small round cells. Note palisade formations. B = palisade formation of the endocardial Aschoff body type. C = considerably widened subendocardium. Note infiltration with inflammatory cells and hypercapillarization with many capillaries oriented at right angles to endocardial surface. D = myocardium with distended capillaries, and shows mild interstitial infiltration.

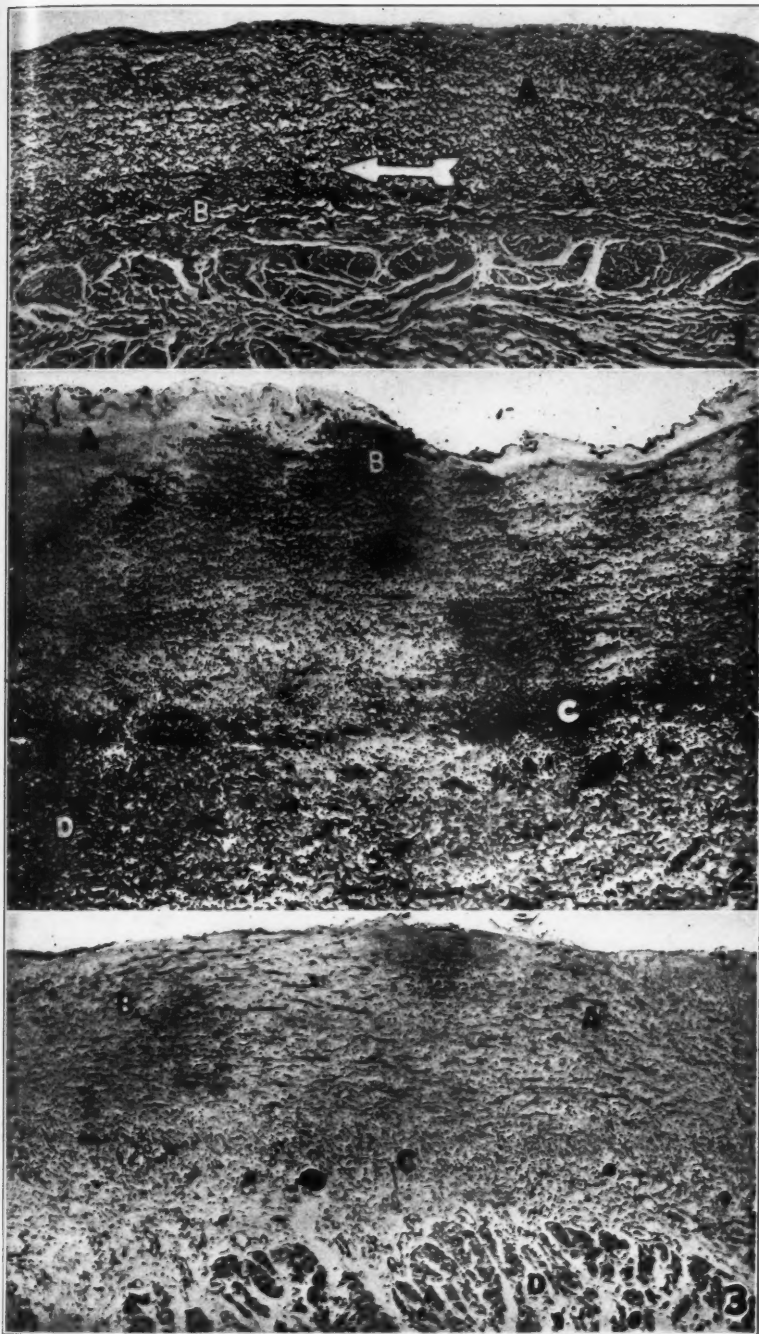


PLATE 101

FIG. 4. Left auricle from active case of rheumatic fever. Age 18 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = endocardial reduplication of edematous type. Note the small round cell inflammatory infiltration of this reduplication and the condensation of the elastic lamellae beneath it. B = endocardium markedly infiltrated with small round cells. Note edema, stretching and separation of elastic fibers. C = markedly inflamed, edematous and widened subendocardium.

FIG. 5. Left auricle from active case of rheumatic fever. Age 12 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = endocardium showing distortion and condensation of elastic fibers; B = condensation of elastic fibers in subendocardium; C = edematous infiltrated subendocardium with hypercapillarization.

FIG. 6. Left auricle from active case of rheumatic fever showing multiple reduplications. Age 13 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = older reduplication of embryonal covered type; B = more recent reduplication of partially covered embryonal type; C = uncovered portion of reduplication; D = endocardium proper showing marked edema, paucity of elastic fibers and mild small round cell infiltration; E = considerable increase in smooth muscle component of outer third of endocardium. Note irregularity of smooth muscle bundles, their separation by connective tissue and F, capillary penetration toward middle third of endocardium. G = markedly widened subendocardium with mild small round cell infiltration and marked hypercapillarization; H = myocardium.



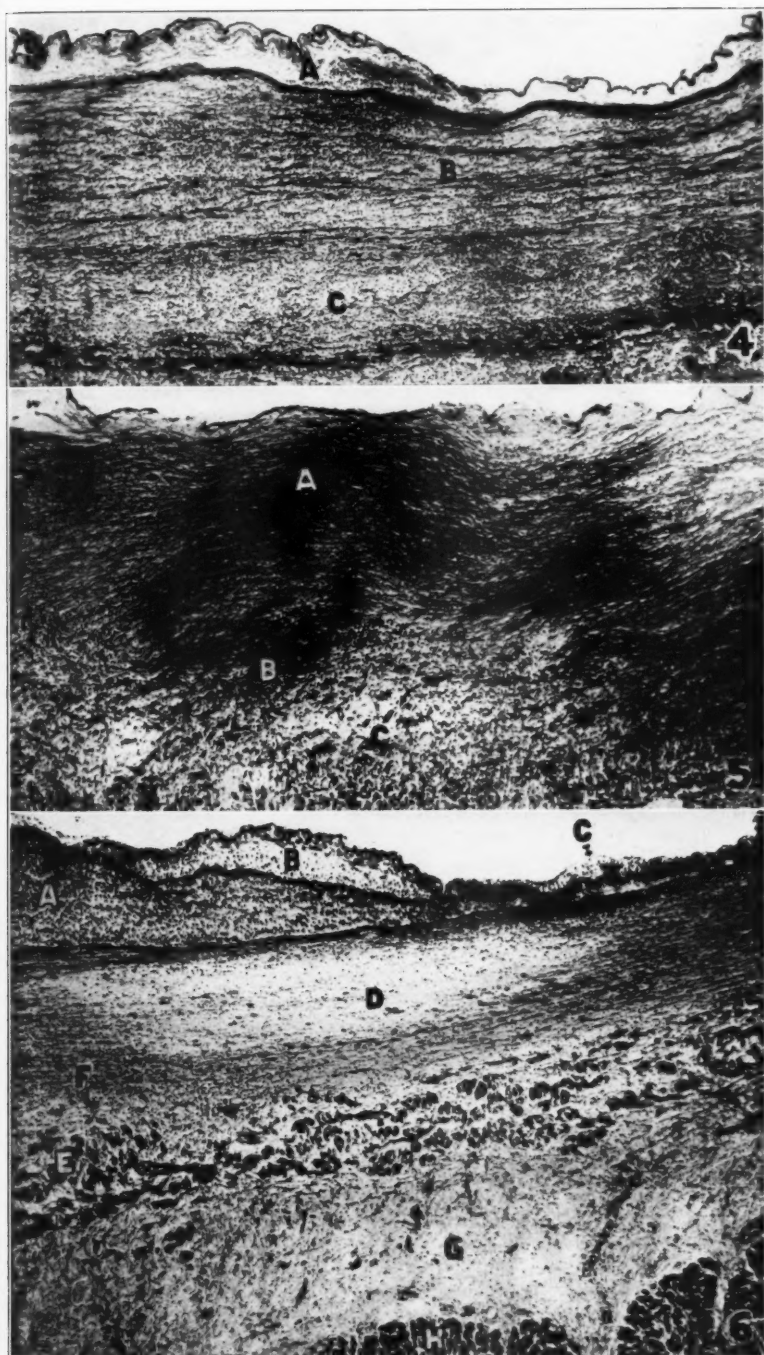


PLATE 102

FIG. 7. Smooth muscle zone in left auricle from active case of rheumatic fever. Age 13 years. High power. Masson's erythrosine-saffron stain. Note considerable increase in smooth muscle cells with irregularity in arrangement.

A = smooth muscle bundle; B = large amount of collagenous tissue between dispersed smooth muscle bundles; C = penetrating capillaries oriented at right angles to endocardium.

FIG. 8. Left auricle from active case of rheumatic fever. Age 15 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = elastified reduplication with suggestion of origin from multiple reduplications; B = inflamed endocardium with capillary penetration to middle third; C = considerably widened, inflamed and hypercapillarized subendocardium; D = myocardium.

FIG. 9. Left auricle from active case of rheumatic fever (injected specimen). Age 7 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = irregular collagenous reduplication with large vascular channels and some small round cell infiltration; B = capillary penetration of endocardium as far as the limiting membrane overlying the inner third; C = middle zone of endocardium showing marked elastica distortion and capillary penetration; D = widened subendocardium with hypercapillarization; E = myocardium.

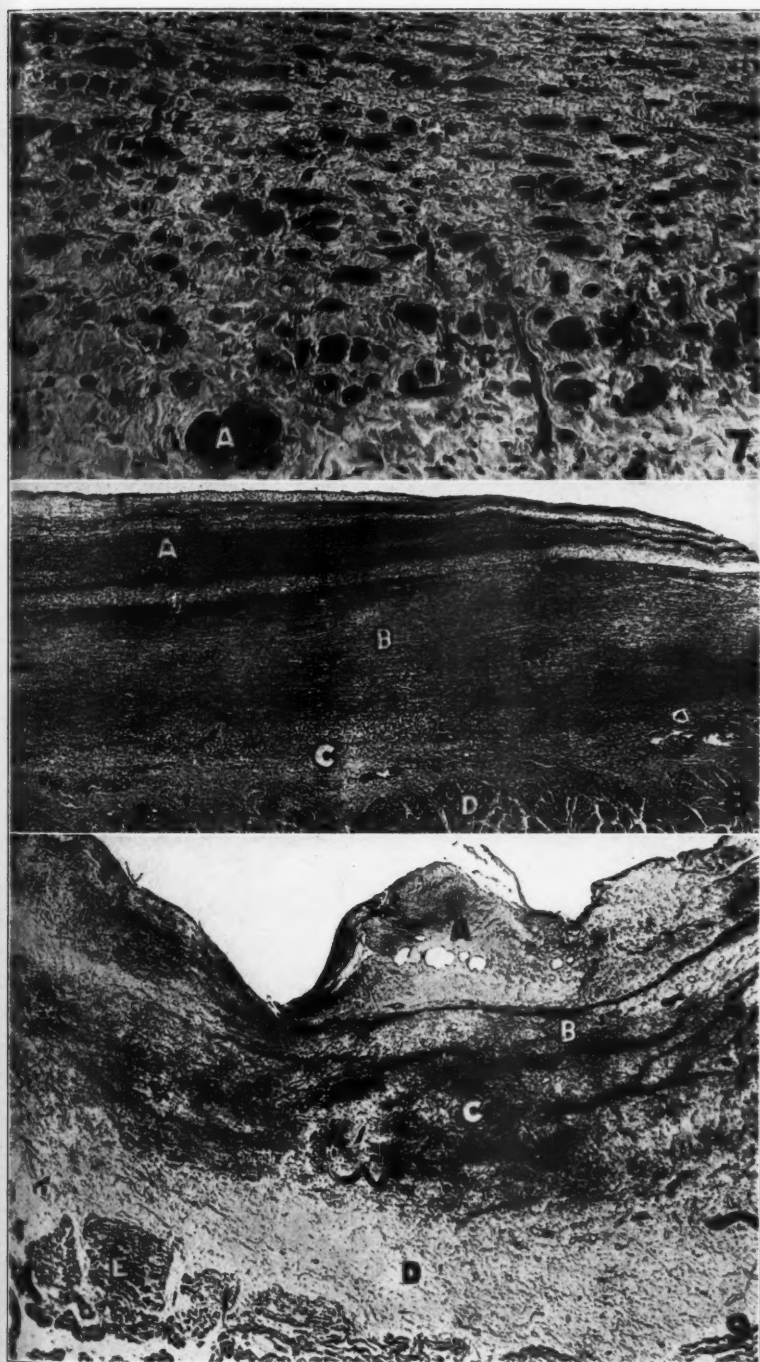


PLATE 103

FIG. 10. Left auricle from active case of rheumatic fever showing multiple reduplications. Age 14 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

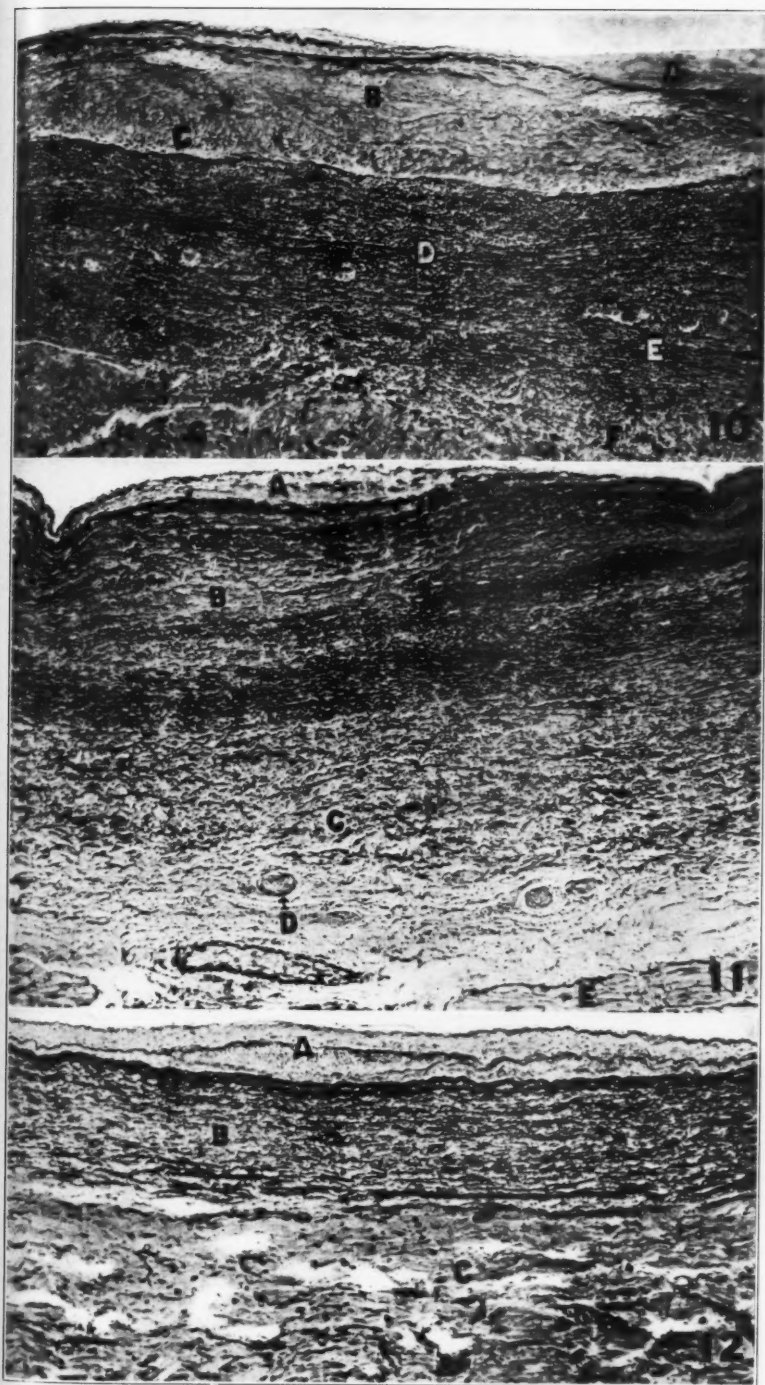
A = most recent reduplication, uncovered; B = older collagenous reduplication; C = smooth muscle component in reduplication; D = endocardium showing marked small round cell infiltration with stretching and separation of elastic fibers; E = penetrating vessels of arteriolar type, in endocardium; F = somewhat widened and infiltrated subendocardium; G = myocardium with mild interstitial infiltration.

FIG. 11. Left auricle from active case of rheumatic fever. Age 39 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = collagenous covered reduplication; B = endocardium showing patchy arrangement of elastic fibers; C = markedly widened subendocardium with moderate small round cell infiltration; D = new formation of subendocardial vessels of the intimal musculo-elastic hyperplastic type; E = myocardium.

FIG. 12. Left auricle from active case of rheumatic fever showing multiple reduplications. Age 49 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = multiple reduplications of endocardium. The reduplication resting on the endocardial limiting membrane is of the covered collagenous type. The innermost reduplication is of the covered embryonal type; B = endocardium; C = myocardium showing marked hypertrophy and some vascular engorgement.





THE VIRUS OF LYMPHOGRANULOMA INGUINALE *

RIGNEY D'AUNOY, M.D., EMMERICH VON HAAM, M.D., AND
LOUIS LICHTENSTEIN, M.D.

(From the Departments of Pathology and Bacteriology of the School of Medicine, Louisiana State University Medical Center, and the Charity Hospital, New Orleans, La.)

CONTENTS

- I. HISTORICAL REVIEW
- II. SOURCES OF VIRUS STRAINS STUDIED
- III. MANIFESTATIONS IN EXPERIMENTAL ANIMALS
 - (a) MONKEYS; (b) WHITE MICE; (c) GUINEA PIGS; (d) OTHER ANIMALS
- IV. CULTIVATION OF THE VIRUS
- V. SUMMARY AND CONCLUSIONS

I. HISTORICAL REVIEW

Discovered nearly 40 years after the important group of virus diseases had been established through the pioneer work of Loeffler and Frosch in 1892 on foot and mouth disease, the virus of lymphogranuloma inguinale, or the "sixth venereal disease" (Stannus), must be regarded as one of the most recent additions to the group of pathogenic filtrable viruses. The first report of apparently successful animal transmission of the disease, known since its classical description by Trousseau in 1865 and excellently described as a clinical entity by Durand, Nicolas and Favre in 1913, was made by Darré and Dumas in 1921. Two of their 4 rabbits, 24 to 48 hours after inoculation with a few drops of pus from the inguinal bubo of a typical case of lymphogranuloma inguinale, into the anterior chamber of the eye, developed a slight iridocyclitis, which during the next 8 days progressed into a severe purulent panophthalmitis, accompanied by severe constitutional symptoms. Cultures of the exudate from the eye proved to be sterile and the animals soon completely recovered.

In the following years (1922-1924) Ravaut and his co-workers observed evanescent inguinal buboes in guinea pigs following the subcutaneous injection of pus from lymphogranuloma inguinale buboes into the region of the groin in a small percentage of their cases. They also confirmed the results obtained by Darré and

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Dumas in rabbits. Most investigators of this period, however, failed to produce lesions in animals and the few apparently positive results met with much adverse criticism (Favre, and Hellerström and Wassen). The diagnostic difficulties which made proper selection of true cases of lymphogranuloma inguinale for experimental purposes often impossible may, in our opinion, account for many of the discouraging results of this period, and we agree with Stannus that many of those doubtful results can be regarded as positive in the light of later experimental evidence.

With discovery of the specific diagnostic skin reaction for lymphogranuloma inguinale by Frei (1925), naturally most of the diagnostic difficulties of the disease were overcome. Shortly after, Hellerström and Wassen reported successful transmission of the disease to monkeys. In 1931 Levaditi, Ravaut, Lépine and Schoen in Paris, and Hellerström and Wassen in Stockholm, independently described a filtrable virus as the causal agent of the disease, both reports appearing in the same journal (*Compt. rend. Soc. de biol.*, 1931, 106) only a few pages apart. In the following years Levaditi and his associates, as well as the Swedish workers and many other continental scientists, continued investigations of the disease and were able to disclose numerous characteristics of the new virus.

Although the disease has been reported in the United States in its various manifestations on numerous occasions and appears to be rather common in this country, with its large negro population, the fact that comparatively little investigation had been made of its causal virus gave us the incentive to study a series of cases occurring in New Orleans. We have been able not only to isolate the virus in seven instances from excised buboes, but also to preserve its virulence by passage through animals. The 160 cases of our series, observed in the short period of 6 months (May–October), were in the majority negroes who had lived all their lives in New Orleans or its immediate vicinity, the possibility of any being infected with an imported virus strain, as observed by Wilmoth, being very remote. Our seven endemic strains of the virus have been rather critically studied and we wish in this report to record some of the data so secured. To facilitate comparison with other isolated strains these data will be presented in close correlation with a critical review of the literature.

II. SOURCES OF VIRUS STRAINS STUDIED

Table I presents data from the case history of each patient from whom we isolated virus strains used in our investigations.

TABLE I
Data on Patients From Whom Virus Strains Were Obtained

Strain No.	Patient	Sex	Race	Age	Significant clinical data	Penile sore	Frei reaction
L 20	J. J.	M	Negro	29 yrs.	Large, bilateral, tender buboes of 3 weeks duration in the inguinal region	None observed	Strongly positive
L 21	M. D.	M	Negro	17	Unilateral, firm, fluctuant bubo of 6 to 8 weeks duration adherent to the skin of the inguinal region	None observed	Strongly positive
L 24	S. J.	M	Negro	16	Unilateral, hard, tender bubo of 1 weeks duration in inguinal region	None observed	Positive
L 26	P. F.	M	White	18	Large, unilateral, fluctuant bubo of 1 months duration in inguinal region	None observed	Strongly positive
L 27	J. J.	M	Negro	16	Large, firm, unilateral mass of 4 weeks duration in inguinal region showing distinct fluctuation	None observed	Strongly positive
L 31	S. B.	M	Negro	23	Large, tender, fluctuant mass of 1 months duration in right inguinal region; burning sensation in urethra before onset of bubo	None observed	Strongly positive
L 32	W. M.	M	Negro	22	Large, fluctuant mass of 1½ months duration in inguinal region; bubo adherent to the skin of inguinal region from which drained a thick yellow exudate after surgical incision	None observed	Strongly positive

III. MANIFESTATIONS IN EXPERIMENTAL ANIMALS

Monkeys, sheep, rabbits, guinea pigs, white mice, chickens and frogs were inoculated by various routes with the isolated strains.

Characteristic lesions were observed in monkeys, sheep, guinea pigs and white mice, while rabbits, chickens and frogs proved refractory to the virus. Passage from animal to animal was performed with homologous and heterologous species of susceptible animals without any difference in the number of "takes" obtained.

(a) *Monkeys*: Two *Macacus rhesus* and 3 common marmosets (*Hepale penicillata*) were inoculated with material derived from patient J. J., (virus strain L 20). Rhesus monkey A received intracerebrally 0.1 cc. of 20 per cent inguinal gland emulsion prepared from material secured by surgical excision; rhesus monkey B received 0.5 cc. of a similar emulsion into the prepuce. The 3 marmosets were inoculated with 0.1 cc. of 20 per cent brain emulsion obtained from a mouse injected intracerebrally 8 days previously with 20 per cent inguinal gland emulsion.

The rhesus monkeys during 6 months of observation did not show any local or general symptoms of disease. The 3 marmosets began to show signs of illness 5 to 9 days after inoculation. The first characteristic symptoms consisted of loss of appetite and general muscular weakness, soon followed by increased muscular irritability and epileptiform convulsions. One to 3 days after onset of symptoms paralysis of the extremities was noted, usually appearing first in the hind legs. Finally, the monkeys became comatose and died 7, 9 and 13 days, respectively, after inoculation. Autopsy showed essentially slight hyperemia of the brain and meninges.

Microscopic study of sections from the brain and cord with their meninges showed dense meningeal infiltration with an increased cellular exudate. The cell types were mostly young large lymphocytes and large mononuclear cells (monocytes), neutrophilic leukocytes and plasma cells being distinctly in the minority, but more apparent however in the animal that died 7 days after inoculation. The meninges of the convex side of the brain and those at the base were equally affected, the exudate being especially marked in the deep sulci. The meninges of the ventricles showed similar infiltration. The nervous elements of the brain and of the spinal cord showed no appreciable changes. Some of the ganglia cells near the sites of infiltration were slightly pyknotic and appeared somewhat shrunken, but no absolute evidence of degeneration could be observed. The Virchow-Robin spaces of a large number of vessels of the brain and of the spinal cord showed dense perivascular, collar-

like infiltration with lymphocytes and large mononuclear cells. In a future report these pathological findings will be described in more detail and compared with changes evoked by other filtrable viruses.

Antigens prepared after Frei's technic with 20 per cent infected brain emulsion in sterile saline solution gave positive skin reactions in patients afflicted with lymphogranuloma inguinale. The advantages of this method of preparing the antigen for the diagnostic skin test have been emphasized (von Haam and Lichtenstein) on previous occasions.

Intracerebral inoculation of 20 per cent saline emulsions of infected monkey brains in mice produced typical lesions. Transmission of the virus to mice could also be effected by intracerebral inoculation of heart's blood and 20 per cent spleen emulsion from infected monkeys, but not by similar injections of liver and kidney emulsions.

Our observations on the manifestation of the virus of lymphogranuloma inguinale in the monkey agree, except in few instances, with the experiences of the European workers. The difference of susceptibility of the various species of monkeys, as stressed first by Hellerström and Wassen, and Levaditi and co-workers, was well demonstrated in our small series of animals, both rhesus monkeys remaining resistant to the virus. Similarly, Findlay succeeded in infecting only 3 out of 10 rhesus monkeys.

Thus far none of our infected monkeys have recovered after showing signs of the disease, and we were unable to study serum protective or virucidal action of such serums as has been done by Findlay, Levaditi and co-workers. The presence of the lymphogranuloma inguinale virus in other organs than the brain following intracerebral inoculation had been previously demonstrated by Hellerström and Wassen, and Levaditi and co-workers, who were able to transmit the disease with heart's blood and splenic emulsions obtained from intracerebrally infected animals. Levaditi also emphasized the possible passage from monkey to mouse and again to monkey. With us this has proved an excellent method of preventing "autosterilization of the virus" observed after continuous passage through monkeys or kittens.

None of our animals showed degeneration of the posterior tracts of the spinal column with demyelination of the nerve fibers and axis cylinder changes, as reported by Jonesco-Mihaiesti and co-

workers. Levaditi and Mollaret in a recent publication believe that the changes reported by these Roumanian workers are the results of spontaneous lesions described by him and others some years ago.

Positive Frei reactions with brain emulsions of infected monkeys were obtained by Hellerström and Wassen, Cohn and Kleeberg, and the pathogenicity of unheated brain emulsions for man was demonstrated in a single experiment by Levaditi and his co-workers. The interesting observation of Bonne and co-workers in Dutch East India that heated monkey brain antigen produced in three volunteers typical axillary paradenitis is believed by Stannus to be due to faulty technic in antigen preparation and calls for more confirmation. This we have not been able to do.

(b) *White Mice*: Because of the enthusiastic reports of Findlay and Wassen, we employed white mice extensively in transmission experiments. In the experiments herein reported we made use of 345 mice, 85 serving as controls. All animals came from a well known source and were carefully observed in isolation cages before use. Intracerebral injections were performed with a very fine short needle (26 gauge, $\frac{1}{4}$ inch), the material injected (inguinal bubo pus from human cases or 20 per cent infected brain emulsion in saline) never exceeding 0.01 cc. For hours after injection most animals behaved normally, the incidence of serious traumatization of brain substance by injection being negligible. All seven virus strains were used in mice experiments and were mostly kept virulent by means of numerous animal passages. Strain L 20 was carried at the time of presentation of this report through 17 passages; strain L 26 through 11 passages; strain L 27 through 11 passages; strain L 31 through 9 passages; and strain L 32 through 7 passages. Strains L 21 and L 24 lost their pathogenicity for mice after the 3rd passage. With strain L 20 we attempted to determine the most advantageous interval between inoculations in order to maintain optimum virus virulence. Accordingly, groups of mice were inoculated at weekly, biweekly, and monthly intervals and the comparative strength of the virus estimated from the percentage of "takes," the time of appearance and severity of clinical symptoms and the histological pictures of the lesion produced. Weekly or biweekly intervals were found best for virulence-sustaining inoculation, monthly intervals generally leading to decrease of virus virulence. This can be explained by the fact that only more resistant or less

infected animals survive as long as 4 weeks after inoculation with the virus; subsequent transmissions from such animals will then be made with brain material containing less virus or more immunizing substances. We routinely use biweekly intracerebral mouse inoculations in carrying our strains.

The incubation period in white mice was characterized by wide individual variations. Some of the animals showed symptoms as early as 4 days after intracerebral injection, while others were apparently perfectly well at the time they were killed for scheduled passage of the virus to other animals. Animals that died 1 to 3 days following injection were regularly discarded, as in such cases trauma from the injection played too large a factor to allow correct conclusions. In the majority of such cases the animals had shown severe shock following injection, with paralysis of one or more extremities occurring for several hours.

The first symptoms attributable to the virus in white mice following intracerebral injection were weakness, loss of appetite and roughened appearance of the fur; the animals moved slowly and listlessly and lost weight. Later, nervous symptoms became manifest. These consisted of muscular weakness with paralysis of the extremities, especially the hind legs; epileptiform movements and sometimes chronic convulsions occurring in short attacks. The typical "encephalitis position," as described by Fischl and Schaefer in experimental herpes encephalitis in mice, was observed in several instances. Toward the later stages severe unilateral or bilateral conjunctivitis was usually noted, with hemorrhagic exudate covering the cornea. Animals showing this symptom complex usually died in 3 to 5 days after its onset; none recovered spontaneously.

Autopsy and histological studies of infected mouse brains showed lesions similar to those described in the brains of infected monkeys. In all infected mice the typical histological changes could be observed as early as 4 days after intracerebral inoculation, being independent of clinical symptoms.

The microscopic picture of the lesion was that of an extremely cellular exudate consisting of lymphocytes and large endothelial cells around the vessels of the meninges. In some few cases more fibrinous exudate and a larger number of neutrophilic leukocytes were noted than were observed in the brains of similarly infected monkeys. In animals killed 1 month after inoculation, without the

advent of nervous symptoms, the infiltration was limited to a more or less circumscribed collection of cells, mostly small lymphocytes, around the larger vessels of the meninges — so-called "lymphomas."

Except for the very weak virus strains (L 21 and L 24), we were able indefinitely to reproduce the same type of lesions by repeated intracerebral inoculations of 20 per cent infected brain emulsions. With such infected mouse brain emulsions we were also able to produce typical lesions in monkeys, sheep and guinea pigs, but not in chickens or frogs.

The antigenic value of infected mouse brain emulsions, as determined by the Frei test, was generally as high as that observed with emulsions of infected monkey brains. Spleen and kidney emulsions of intracerebrally infected mice also gave positive Frei reactions in patients with lymphogranuloma inguinale; such reactions, however, were much weaker than the ones produced with brain emulsion from the same animals.

In Findlay's mice experiments considerable variation in incubation time was noted. For his 586 mice that died of fatal meningo-encephalitis produced by intracerebral inoculation of lymphogranuloma inguinale virus, the incubation period was between 5 and 94 days, with an average of 34 days. Contrary to this observation, Grace and Suskind report an incubation period of 2, 4 and 7 days in their passage experiments with a virus strain isolated in New York. Our experience in this respect agrees more with that of Findlay. However, with many of our mice no signs of disease were noticeable when the animals were killed for routine transmission of virus 2 weeks after infection. The difference between the results obtained by Grace and Suskind, and Findlay and ourselves may be caused by varying virulence of the viruses used, a factor stressed by Levaditi and his co-workers. Another contributing factor may be the dosage of inoculated material. In Findlay's report no data concerning dosage are given. Grace and Suskind injected 0.03 cc. of 20 per cent and 40 per cent brain emulsions, which is considerably more than the dosage used by us. Although their control animals did not show any detrimental effect from this dosage, it is possible that the higher degree of traumatization of the brain accelerated the effects of the virus. Such combined effects of brain trauma and remote inoculation with virus of lymphogranuloma inguinale have been reported by Findlay, who obtained localization of the virus in the

brain after intraperitoneal injection of the infectious material, the brain having been previously traumatized by injection of sterile starch solution. In the course of our transmission experiments we received the impression that the virulence of some of our strains for mice increased with successive passage; our observations, however, do not point as strongly in this direction as do those of Grace and Suskind.

The histological picture of the experimental meningo-encephalitis produced by inoculation of lymphogranuloma inguinale material must be differentiated from the picture of spontaneous encephalitis in white mice, as described in this country by Cowdry and Nicholson. In this condition infiltration of the meninges, the perivascular spaces of the brain and the subependymal areas with round cells is also seen; the type of infiltration, however, is more focal and the cell type mostly the small lymphocyte. Cowdry and Nicholson, who described 25 cases of spontaneous encephalitis among 141 stock mice, emphasized that the animals showed no symptoms of disease and could not be differentiated from healthy ones. They observed a protozoan-like parasite similar to the *Encephalitozoon cuniculi* (Levaditi) in brain sections of these animals. In reporting experiments with white mice Levaditi and co-workers emphasized the fact that the virus of lymphogranuloma inguinale, similar to *Encephalitozoon cuniculi* or the virus of recurrent fever, could remain in the central nervous system of white mice without apparently harming the animals. We have encountered spontaneous encephalitis in some of our local mice strains and wish to point to the advisability of using strains of mice free from or usually quite resistant to epizootic encephalitis in studying neurotropic viruses. As additional precaution, we considered positive "takes" only such animals whose brain emulsions were able to elicit unmistakably positive Frei reactions in humans suffering from lymphogranuloma inguinale.

(c) *Guinea Pigs*: Many authors report negative or doubtful results in transmission experiments with guinea pigs. In contrast to the many discouraging reports, however, Meyer, Rosenfeld and Anders recorded nearly 100 per cent success in their guinea pig inoculation experiments. They apparently observed typical lesions produced by the virus not only in the regional lymph glands but also in the mesenteric glands, lungs, spleen and liver. Nicolau,

likewise, reported such generalized infection of guinea pigs with the virus. He and Findlay, however, sound a warning that spontaneous lesions in guinea pigs caused by infection with *Pasteurella pseudotuberculosis* may be mistaken for lesions of experimental lymphogranuloma inguinale. In these views we fully concur as a result of our own experiences.

Fourteen guinea pigs were inoculated with infected mouse brain emulsions (strain L 20). Each animal received 0.1 cc. of 20 per cent organ emulsion subcutaneously in both inguinal regions. The slight infiltration following injection disappeared completely after a day. After intervals of from 4 to 8 days, 5 of the inoculated animals developed palpable enlargement of the inguinal glands. These animals were sacrificed and their inguinal regions exposed. The lymph glands were markedly enlarged and of a dark reddish gray color. Surfaces of the swollen glands revealed by sectioning showed small yellow areas suggesting microscopic abscesses. The periglandular connective tissue was injected, the lymph vessels clearly visible. The disease process was always localized in the inguinal glands and no generalized infection, as described by some continental authors, could be observed. Microscopic pictures of the glands showed marked proliferation of the endothelial cells with some giant cells present, together with infiltration with polymorphonuclear leukocytes in the form of small cell collections resembling the microscopic abscesses so typical of inguinal buboes seen in human lymphogranuloma inguinale. Our few attempts at transmitting the disease by means of glands of such infected guinea pigs to other animals have so far failed.

(d) *Other Animals*: Although greatest success is obtained in transmission experiments by the use of monkeys and white mice, numerous other species of animals can be infected with the virus of lymphogranuloma inguinale. Freund and Reiss, and Chevallier and his co-workers report successful transmission to rabbits by intracerebral inoculations. On the other hand, Findlay obtained negative results with 16 rabbits injected subcutaneously in the groin region and similar negative results following infection by the intracerebral route. He concludes, however, that the virus may remain virulent in the central nervous system of rabbits for at least 10 days. Levaditi, Ravaut, Schoen and Vaisman successfully infected cats; Nicolau and Findlay, dogs. In the brains of field moles the virus

retains its virulence for white mice for 30 days without apparently harming the intermediate host.

We inoculated 2 sheep (mother and young) intracerebrally with 0.5 cc. of a 20 per cent pooled brain emulsion of infected mice. The lamb died after 5 days. Histological studies showed typical meningo-encephalitis. Twelve young chickens (white Leghorn) inoculated intraperitoneally and intracerebrally with a very infectious pooled mouse brain emulsion remained symptomless with no demonstrable histological lesions. White mice, however, injected intracerebrally with such infected chicken brain emulsions showed typical meningeal reactions, while control mice injected with normal chicken brain emulsions showed no clinical or histopathological changes whatsoever. This shows, as previously pointed out by Findlay, that the virus is able to survive in the chicken brain for some time without losing its virulence. Eight frogs (*Rana catesbiana* and *Rana pipiens*) inoculated with infected mouse brain emulsions showed no symptoms of disease or histopathological changes in their central nervous systems. Mice inoculated with infected frog brain emulsions remained symptomless and showed no histological changes.

IV. CULTIVATION OF THE VIRUS

All early attempts at cultivating the virus of lymphogranuloma inguinale failed (Stannus). In 1931 Langer, in discussing a paper by Levaditi on this subject, reported apparently successful cultivation of the virus in symbiosis with cell cultures. Of 19 guinea pigs inoculated by him with the second to fifth subcultures of the virus, 10 showed the typical lesions of human lymphogranuloma inguinale, in 4 the histological lesions were "doubtful," and 6 showed no changes or only a non-specific inflammatory reaction. Seven control animals showed no histological changes or only non-specific inflammatory changes. The virus cultures did not give positive skin reactions with patients suffering from the disease. Langer conceded that the latter factor and the uncertainty concerning the histopathology of lymphogranuloma inguinale in the guinea pig considerably restricted the scientific value of his results. In 1935 Tamura reported cultivating the virus of lymphogranuloma inguinale in the medium devised by Maitland and co-workers for the cultivation of vaccinia. From 0.02 cc. to 0.03 cc. of diluted pus secured from an

inguinal bubo inoculated into 7 cc. of Tyrode solution containing a small piece of fresh liver or kidney from guinea pigs produced, after incubation from 36 to 48 hours, a peculiar cloudiness of the medium which was transmissible and could be carried on through as many as twenty-four subcultures. With such heated cultures Tamura was successful in eliciting positive Frei reactions in patients suffering from lymphogranuloma inguinale.

Applying Tamura's method, 0.1 cc. of 20 per cent infected mouse brain emulsions were placed in sterile test tubes containing 10 cc. of Tyrode solution and approximately one-third of the kidney of a rabbit starved for 24 hours before death. The medium was usually prepared the day preceding inoculation and incubated for 12 hours to insure sterility. Two to 3 days after inoculation a distinct cloudiness appeared in the tubes inoculated with infected material, while control tubes remained clear. From the second and the third subcultures mice were inoculated intracerebrally with 0.01 cc. of the cloudy fluid. They developed the characteristic histopathological changes previously described. Mice inoculated with material from control tubes 8 days after inoculation showed no signs of any meningeal reaction. Examination of the cloudy Smith-Noguchi-Maitland tubes failed to show bacterial growth. The dilution of the primary mouse virus in the second subculture was 1:10,000. We offer this result as preliminary confirmation of Tamura's work, believing that the characteristic meningo-encephalitis produced in white mice after inoculation of culture material must be regarded as more convincing proof of cultivation of the virus of lymphogranuloma inguinale after Tamura's method than his own evidence derived from guinea pig experimentation.

V. SUMMARY AND CONCLUSIONS

1. Seven endemic strains of the virus of lymphogranuloma inguinale have been isolated and transmitted to animals.
2. Intracerebral inoculation of infectious material produced a typical meningo-encephalitis in the common marmoset while the rhesus monkey proved resistant to such inoculations.
3. The virus could readily be transmitted to white mice, biweekly inoculations allowing upkeep of its maximal virulence.
4. Brain emulsions of infected monkeys and mice act as excellent

stable and sensitive antigens for the specific diagnostic intradermal reaction of Frei.

5. Twenty-eight per cent of infected guinea pigs showed enlargement of the regional lymph glands with histological lesions consistent with the disease.

6. Experiments with sheep, chickens and frogs indicate that the virus can infect sheep; that its virulence can be preserved in the brains of chickens; and that frogs cannot be infected.

7. Cultivation of the virus after the method of Tamura was possibly confirmed.

8. The virus of lymphogranuloma inguinale, as encountered epidemically in the poorer negro population of Louisiana, shows rather identical behavior concerning animal transmission as the virus strains studied in other parts of the United States and abroad.

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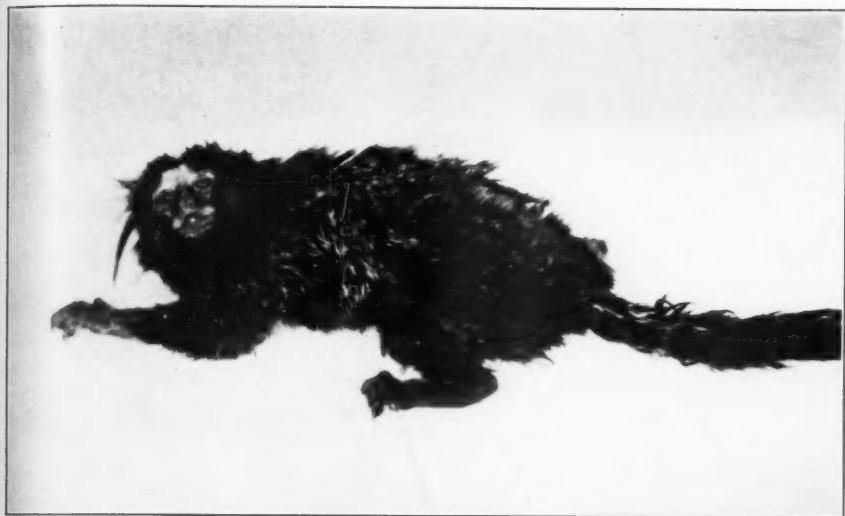
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DESCRIPTION OF PLATES

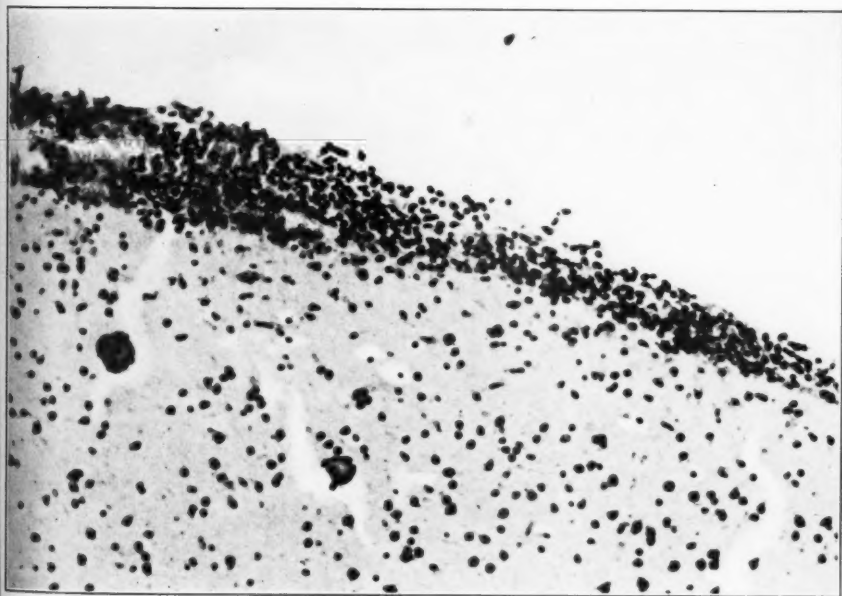
PLATE 104

FIG. 1. Intracerebrally infected monkey (*Hepale penicillata*). Complete paralysis with convulsions 9 days after inoculation.

FIG. 2. Meningo encephalitis in infected monkey (*Hepale penicillata*). Dense infiltration of meninges with small round cells. Hematoxylin-eosin stain. $\times 120$.



1



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PLATE 105

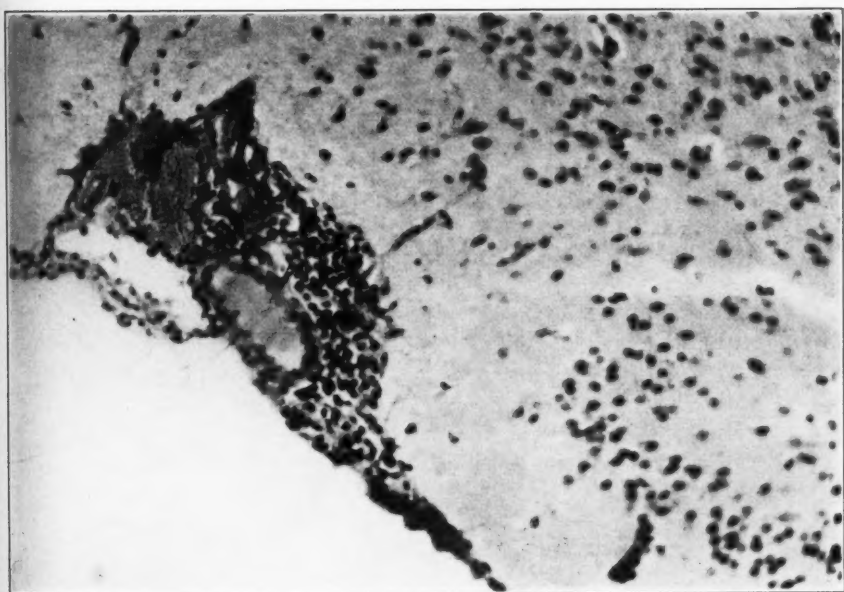
FIG. 3. Intracerebrally infected white mouse. Severe paralysis with convulsions 11 days after inoculation.

FIG. 4. Meningo-encephalitis in white mouse. Focal infiltration of meninges with round cells ("lymphoma"). Hematoxylin-eosin stain. $\times 120$.





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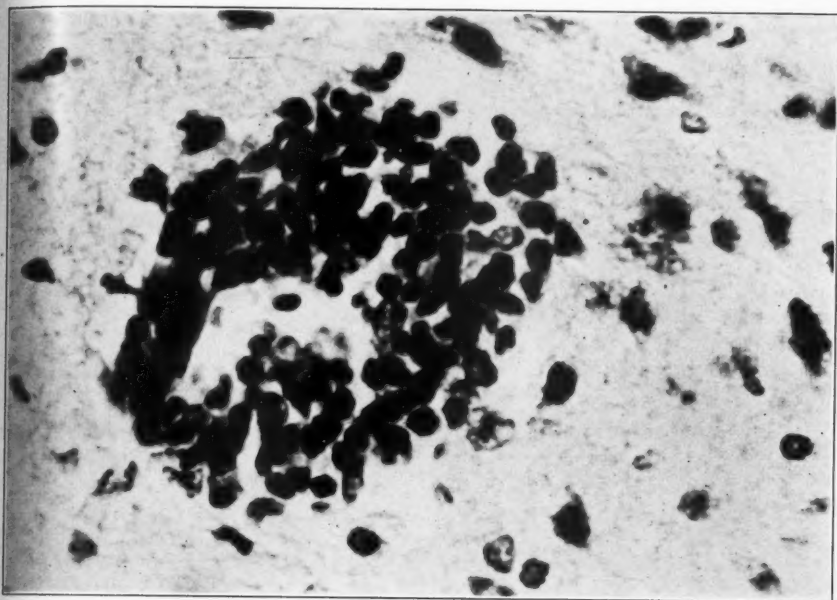
D'Aunoy, von Haam and Lichtenstein

Virus of Lymphogranuloma Inguinale

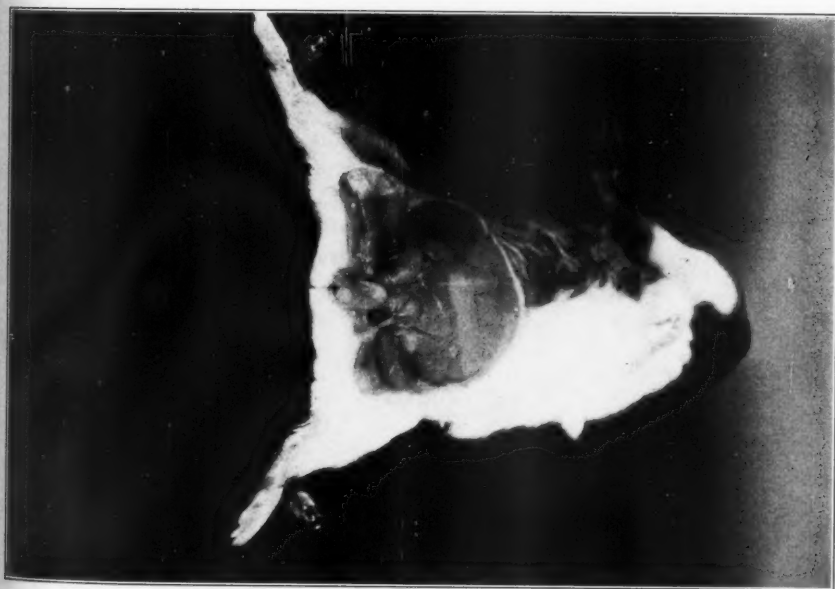
PLATE 106

FIG. 5. Meningo-encephalitis in white mouse. Perivascular round cell infiltration of Virchow-Robin space of the brain. Hematoxylin-eosin stain. $\times 450$.

FIG. 6. Subcutaneously infected guinea pig. Enlargement of regional glands 8 days after inoculation.



5



6

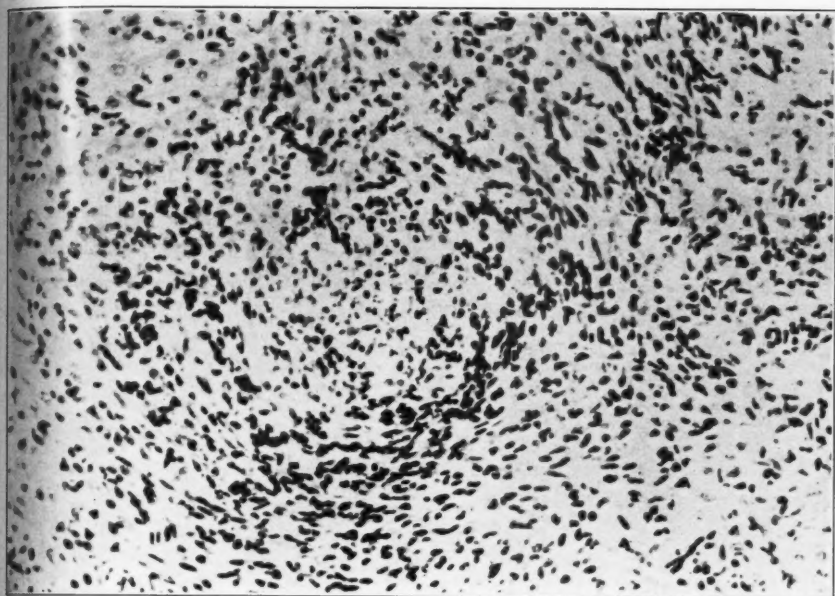
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Virus of Lymphogranuloma Inguinale

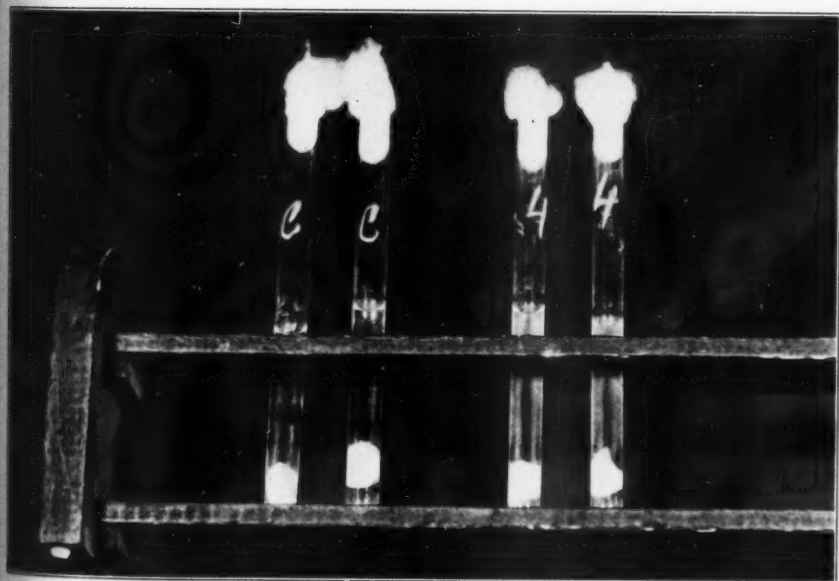
PLATE 107

FIG. 7. Acute bubo in guinea pig. Numerous endothelial cells and polymorphonuclear leukocytes. Hematoxylin-eosin stain. $\times 120$.

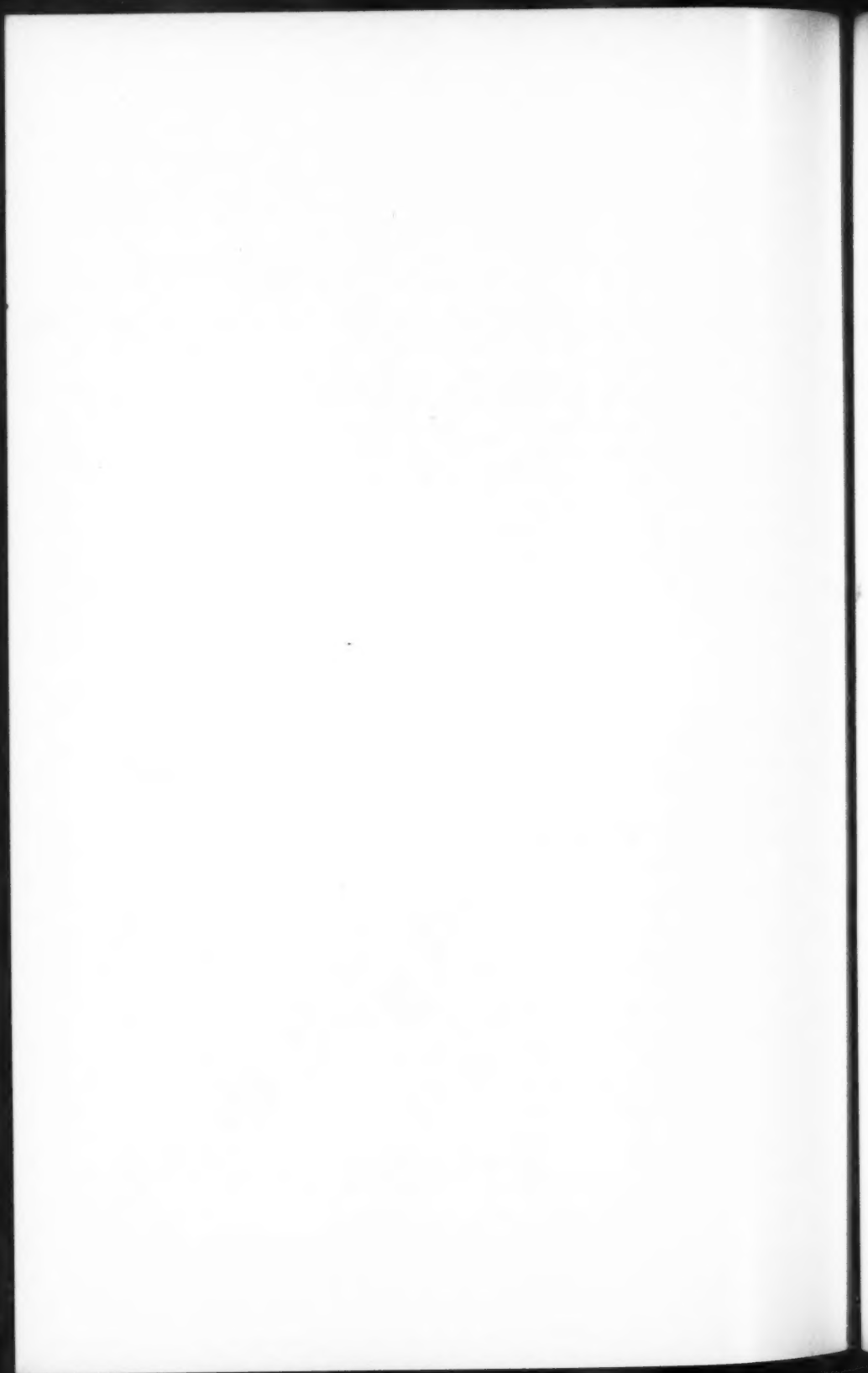
FIG. 8. Cultivation of the virus after Tamura. Note the cloudiness of the inoculated Maitland media.



7



8



RHABDOMYOSARCOMA OF THE PROSTATE *

F. H. FOUCAR, LT.-COL. M.C.

(From the Laboratory Service of the Walter Reed General Hospital, Washington, D. C.)

Tumors arising from striated muscle are rare. When such tumors are composed of well differentiated striped muscle the growth is classified as a rhabdomyoma. Rhabdomyomas are usually benign, sharply circumscribed and of developmental origin. Malignant rhabdomyomas (rhabdomyosarcomas) are composed of undifferentiated striped muscle. Atypical striped muscle cells may form a part of malignant teratoid tumors arising in the kidney, testis, ovary and sacral region. Approximately 50 per cent of the pure type of rhabdomyosarcomas occur in the genito-urinary tract.

Like other malignant neoplasms arising from mesenchyme, rhabdomyosarcomas metastasize by blood stream and regional lymphatics and give early lung and bone involvement. The prostate presents excellent opportunity for blood stream seeding by way of the venous plexi beneath the capsule. The malignancy of rhabdomyosarcoma, as of all newgrowths, is in inverse ratio to the degree of differentiation of the tumor cells.

Malignant rhabdomyomas primary in the prostate are rare. Ewing¹ cites 3 cases, all occurring in young adults. Stout² mentions 1 case of malignant rhabdomyoma reported by Kretschmer. Kretschmer³ in his article, "Sarcoma of the Prostate," presents 1 case of rhabdomyosarcoma, age 31 years. Examination of tissue removed at operation was diagnosed as rhabdomyosarcoma by Dr. A. S. Warthin, of the University of Michigan.

De Rom and Thomas⁴ state that 36 per cent of prostatic sarcomas are of the round cell type and 20 per cent of the fusiform cell type; the 44 per cent remaining include myxosarcomas, lymphosarcomas, angiosarcomas, fibrosarcomas, leiomyosarcomas and rhabdomyosarcomas. They quote Ewing as follows: "The only well defined variety of prostatic sarcoma is the rhabdomyosarcoma." In the case of rhabdomyosarcoma reported the patient was 23 years of age. The article is well illustrated.

* Received for publication April 15, 1935.

Culver⁵ collected 59 cases of prostatic sarcoma from the literature and added 17 more. These 76 cases include 24 of the round cell type, 17 of the spindle cell type, 8 myxosarcomas, 5 lymphosarcomas, 4 rhabdomyosarcomas, 3 angiosarcomas, the remainder being undifferentiated. The case he reports is of the large round cell type.

Greig⁶ reported a case of rhabdomyosarcoma of the prostate in a child aged 4 years. Initial incontinence was unaccompanied by pain, but pain developed later during micturition. A suprapubic cystostomy was performed and "a large fungating tumor, size of Tangerine orange, found." The patient died 10 days after operation. The microscopic diagnosis was rhabdomyosarcoma.

Katzmann⁷ published an article on malignant rhabdomyoma of the prostate in a child. He cited 30 cases of prostatic sarcoma reported up to the time of his article, among which there were 3 cases of malignant rhabdomyoma. His conclusion is that while sarcoma of various types may occur at any age, rhabdomyosarcomas are confined to childhood. This conclusion is not borne out by the findings of other observers.

The records of the Walter Reed General Hospital* include one sarcoma of the prostate occurring in a white male aged 31 years. Microscopic examination of the tissue removed at operation showed a spindle cell tumor, the cells arranged in interlacing bundles. The diagnosis was malignant fibrosarcoma.

References to prostatic sarcoma, type not classified, are numerous. All articles on this subject stress the rarity of the condition, its occurrence during youth and the rapidity of the course. A prostatic sarcoma has a soft, balloon-like feel. Tenderness is frequently absent. Deaver⁸ very aptly states that by the time the patient seeks medical advice the tumor will have reached considerable size, almost completely filling the pelvis. The bladder is pushed up and forward, the bladder wall not invaded. Cystoscopic examination is only valuable in demonstrating an extravescical mass with elevation and distortion of the trigonal area. Boyd⁹ brings out the difficulty in differentiating sarcoma from a highly anaplastic carcinoma. In the differential diagnosis between sarcoma and carcinoma of the prostate it is important to stress the wide difference in age incidence, rapidity and size of growth, and degrees of firmness and tenderness on palpation. The prostatic carcinomas are hard and nodular; the

* Walter Reed General Hospital, Washington, D. C., Reg. No. 53695.

sarcomas are larger and elastic to the touch. Residual urine is usual in prostatic carcinoma and not generally present in sarcoma.¹⁰ Carcinoma of the prostate is frequently encountered while sarcoma is relatively rare. The bone metastases in carcinoma of the prostate are primarily of the pelvic girdle and are of the osteoplastic type; in sarcoma of the prostate the bone metastases are general and are of the osteolytic type. Multiple pulmonary metastases suggest sarcoma.

The prognosis and treatment of sarcoma of the prostate link themselves with the degree of anaplasia, applicable to malignant neoplasms in general.

Briefly reviewing the anatomy of the prostate^{11,12}: the prostate normally measures 4 cm. transversely at base, 2 cm. in its antero-posterior diameter, and 3 cm. in its vertical diameter. Its weight is about 8 gm. It is located around the commencement of the male urethra, below the urinary bladder and presents thirty to fifty compound tubulo-alveolar glands located in the lateral lobes. These glands empty by from sixteen to thirty-two excretory ducts, opening into the urethra along the grooves on either side of the colliculus seminalis. The prostatic acini have no distinct basement membrane. The interstitial tissue is abundant and represents more than one-half the entire mass of the organ. The interstitial tissue is composed of connective tissue, elastic network and abundant smooth muscle arranged in strands. That portion of the prostate lying behind the pubic bone is represented by a wedge-shaped section, whose apex is directed toward the anterior wall of the prostatic urethra. This wedge-shaped section lies between the two lateral lobes and is composed of dense connective tissue traversed by bands of striped muscle fibers. The fibromuscular stroma of the prostate includes large blood vessels and large sympathetic trunks and ganglia. The veins form a plexus beneath the capsule. A primary newgrowth of the prostate may, therefore, arise from any of the above structures, starting from tissue derived from any of the three cell layers of the primitive embryo.

The following case is reported as being typical of prostatic sarcoma. The age of the patient, the subjective and objective clinical findings, the clinical course, termination and autopsy findings, all are true to this type of malignancy of the prostate. The microscopical examinations further differentiated the neoplastic process as a malignant rhabdomyoma.

REPORT OF CASE

Clinical History: G. R. M., a white male carpenter, 26 years of age, born in Indiana, was admitted to the Letterman General Hospital Aug. 12, 1933. The family history was irrelevant. He had the "usual childhood diseases" and typhoid fever during early adolescence; denied venereal infection and the use of alcohol.

Present Illness: Two weeks prior to admission to the hospital the patient, a member of the Civilian Conservation Corps, noticed a burning sensation in the urethra. He became unable to void and was catheterized. It was only necessary to catheterize once, as after passage of the catheter the patient developed frequency of urination and felt as though he were improving. At this time he noticed a bloody discharge from the external meatus. He was transferred to the Letterman General Hospital. On admission he complained of no subjective symptoms.

Physical Examination: This revealed a greatly enlarged prostate, soft and compressible. The diagnosis of the ward surgeon was prostatic abscess complicated by rupture into the ampulla of the rectum. A blood culture was sterile.

Laboratory Data: Blood chemistry showed urea nitrogen 96 mg., and creatinine 2 mg. per 100 cc. Urine examinations showed a heavy trace of albumin, many pus and red blood cells. Wassermann negative.

Course of Illness: On September 7th cystotomy was performed. The prostate was found to be extremely large and soft, protruding into the lumen of the bladder over the trigonal area. An attempt was made to find the suspected abscess without result. The patient died Sept. 11, 1933.

The course of the case, from the onset of the first subjective symptom (urethral burning) until death, was only 44 days. The autopsy findings are so generalized and massive that it is not fair to assume that the exploratory cystotomy hastened death.

AUTOPSY FINDINGS *

The body is that of a white male, 26 years of age, 165 cm. in length, weighing approximately 50 kg. (110 pounds). Moderate emaciation is present. There is massive neoplastic infiltration of the left, deep cervical lymph nodes which push the larynx and trachea to the right. The thyroid weighs 23 gm. and presents one small metastasis. The posterior bronchial and hilum nodes, bilateral, are massively infiltrated. Both lungs present sharply outlined nodules, varying in size from 0.5 to 2 cm. in diameter, located beneath the pleural surfaces of all lobes. The outer surfaces of these nodules are flattened, smooth and of mushroom shape. The cut surfaces of the lungs present many metastatic nodules located throughout the cortical portion of all lobes. The heart is free from metastatic involvement. There is no peritoneal involvement. The liver weighs 2324

* Autopsy No. 1367, Letterman General Hospital, San Francisco, Calif.

gm.; its surface is studded by circular, slightly raised areas from 0.5 to 2 cm. in diameter. The cut surface of the liver presents a few spherical nodules. As no X-rays had been taken, only the more massive bone metastases are noted, osteolytic in type, including two of the calvarium and one replacing the body of the twelfth dorsal vertebra. The kidneys show the picture of a chronic, suppurative (ascending) pyelonephritis, more advanced in the left kidney. The spleen weighs 342 gm. and is of the septic type.

The *urinary bladder* shows an operative incision through the antero-inferior wall closed by interrupted catgut sutures, excepting the upper angle which admits a rubber tissue drain into the bladder lumen. The bladder wall is contracted, the mucosa presenting a hemorrhagic, ulcerative reaction. The ureteral orifices are obscured by hemorrhage and edema of the surrounding mucosa. The trigone is replaced by a large, soft, pale pinkish gray mass, the summit of which shows a transverse laceration (made at the time of exploratory cystotomy).

The *prostate* is 11 cm. in diameter. The anterior and lateral aspects of the prostatic mass are relatively firm and their cut surfaces are pinkish gray, smooth and homogeneous. The posterior aspect is necrotic, reduced to a grayish brown substance showing central liquefaction. The *newgrowth* replaces the entire prostate and has destroyed the seminal vesicles and ampullae of the vasa deferentia, and infiltrates the rectovesical space, fungating through the adjacent wall of the rectum. The rectum wall presents a circular orifice with a necrotic edge. The prostatic urethra is elongated, its lining surface necrobiotic, the normal markings obliterated. The *lymph nodes* lying upon the external iliac vessels are greatly enlarged, on the right side forming a globular mass 4 by 6 cm. The infiltrated nodes are soft and necrobiotic.

Microscopic Appearance of the Newgrowth: The tumor is extremely cellular, composed of spindle cells with large oval nuclei and small nucleoli. The cell outlines are poorly marked. Mitotic figures are numerous and atypical. There are many areas of hemorrhage. In the lung the newgrowth fills the lumens of the aveoli and extends into the lumens of the veins. The rapidity of this growth is shown in the liver metastases where the death of the invaded parenchyma does not keep pace with the neoplastic infiltration. Careful study of the cell morphology of the newgrowth presents: (a) scattered cell nests,

composed of more highly differentiated cells presenting varying amounts of acidophilic cytoplasm with sharp cell outlines; (b) spindle cells, the acidophilic cytoplasm of which shows both cross and longitudinal striae; (c) large, round, oval and giant cells presenting well differentiated striae, both cross and longitudinal, massed in the periphery of the cell body; (d) large cells showing an unstained perinuclear zone crossed by fibrils, producing a spider-like appearance. The illustrations reveal the minute architecture of the more highly differentiated cells, malignant variants of striped muscle.

CONCLUSIONS

Prostatic sarcoma is rare but its possibility must be considered when there is prostatic enlargement, even in adult life. The differential diagnosis between sarcoma and carcinoma of the prostate is made by rather wide differences: (a) in age incidence; (b) in rapidity of the growth; (c) in consistence of the growth; (d) by the absence or presence of local tenderness; and (e) by the location and character of the bone metastases.

Among the various types of sarcoma encountered, rhabdomyosarcoma offers cell detail clearly denoting the parent cell from which the growth originated.

NOTE: I desire to express to the Curator of the Army Medical Museum my appreciation of the splendid results obtained in taking the photomicrographs.

Dr. Arthur Purdy Stout of Columbia University was kind enough to express his opinion regarding this case.

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DESCRIPTION OF PLATES

PLATE 108

FIG. 1. Lung metastasis showing a fusiform cell presenting cross and longitudinal striae and larger cells showing perinuclear rarefaction. Hematoxylin-eosin. $\times 2160$.





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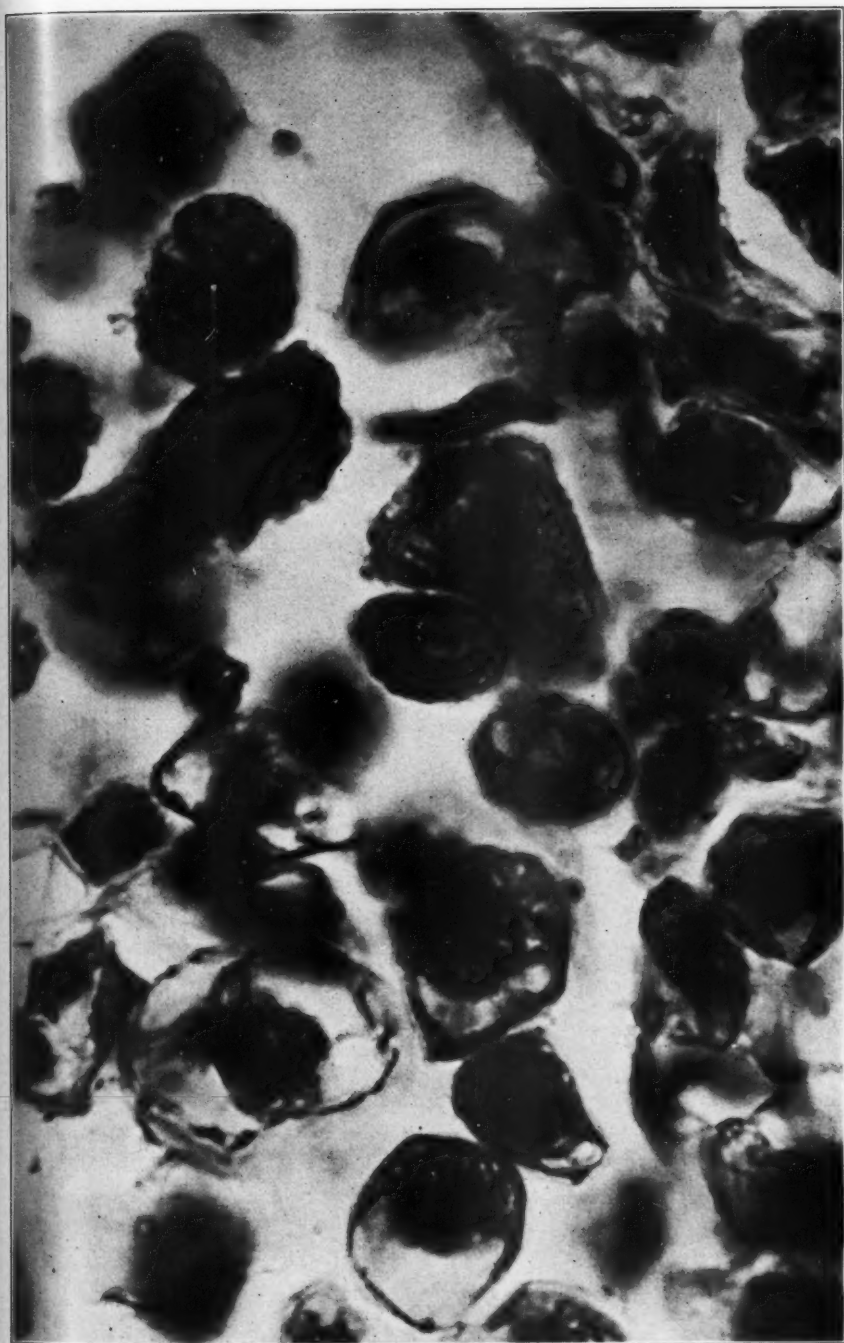
Foucar

Rhabdomyosarcoma of Prostate

PLATE 109

FIG. 2. Lung metastasis showing giant cells with deep rims of striated cytoplasm and larger giant cells of spider type. Hematoxylin-eosin. $\times 2160$.





2

Foucar

Rhabdomyosarcoma of Prostate

AN ANENCEPHALIC MONSTER WITH "RHINODYMIE" AND OTHER ANOMALIES *

SAMUEL B. BRODER, M.D.

(From the Hull Laboratory of Anatomy, University of Chicago, and the Department of Neuropsychiatry, College of Medicine, University of Illinois, Chicago, Ill.)

This report concerns an anencephalic monster with "Rhinydymie" (duplication of the nose and mouth), spina bifida and diaphragmatic defects associated with malposition of the viscera.

Anencephaly, a not infrequent malformation of the nervous system, is characterized by the absence of both cerebral hemispheres and usually a dark red mass of vascular tissue replacing the calvarium.

Scientific approach toward the problem of monsters began as early as the seventeenth century and became especially prominent in the eighteenth century.

In the early part of the nineteenth century Meckel suggested that monsters might be explained on an embryological basis. Then there followed a period during which pressure and other mechanical factors were looked upon as causative agents; in later years chemical factors were blamed. Later, von Recklinghausen attributed anencephaly to arrested closure of the primitive neural groove, a view generally accepted at present. Today the question may be raised whether teratogenesis can be explained on such environmental or hereditary bases. Jordan postulated that the development of monsters is the result of both heredity and environment and said, "... perfect development would require that both be perfect. Various degrees of imperfection or unfavorableness, in either or both, result in the endless degree of variations, anomalies, malformations, and monstrosities."

Nañagas, who studied 57 cases of anencephaly (43 females and 12 males, and 2 of an undetermined sex), found that in comparing body dimensions of normal with anencephalic fetuses the latter had a characteristic growth rate disturbance which resulted in body pro-

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portions that were almost constant for all anencephalics. He concluded that some factor during intra-uterine life caused the body to assume abnormal proportions, but once these were assumed the body resumed its normal growth.

Experimental embryology has contributed evidence in regard to the factors in the production of monsters. Particularly significant are the experiments of Stockard, Child, and their students, on the effects of anesthetics on the developing embryo. Child's gradient hypothesis, originally introduced in 1915 and referring to the fact that organs which are developing most rapidly at the time of interference suffer most, is still valid.

Anomalies of the endocrine glands are frequently found in monsters but the exact relationship is not understood. Mattina reported 3 cases of anencephaly with changes in the endocrine glands and concluded that thyroid deficiency is in part responsible. Although normal suprarenals in monsters were reported by Barlow, Browne is of the opinion that the adrenals may be entirely absent in anencephaly. According to him the zona fasciculata, which in the full term infant represents only a small fraction of the total cortex, may be as broad in the anencephalic as in the adult. He concludes that the latter results are due either to a failure of development or to the destruction of the pituitary body.

Ettinger and Miller found adrenals absent in only 2 of their 9 cases. In the cases where they were present the disturbance was confined to the cortex, the medulla appearing normal microscopically. Only 8 of the 9 cases studied had an anterior lobe of the pituitary body, and of those only 3 showed a pars nervosa. Josephson and Waller reported similar findings.

Kohn found the pituitary present in all of 11 fetuses but in none was it normal. The pars anterior was invariably present, the pars intermedia absent and the pars posterior was found in only 3 cases. He concluded that the small adrenals were a direct result of the malformation of the hypophysis.

Kiyono in 1925 found the pituitary gland in only 7 of 11 fetuses, and of these only 3 had a pars posterior. The adrenals weighed under 1 gm.

In 1919 Hofstätter demonstrated a definite increase in the size of the adrenals after pituitary injections.

The incidence of monsters is not definitely known. According to

Bean, of every 100 pregnancies 80 end in normal births, 7 are aborted as pathological ova, 12 are aborted as embryos or fetuses showing various degrees of abnormality, and 1 pregnancy results in a monster. Tracy quotes Spangler, who studied 11,521 births and reported the incidence of anencephaly as 1 in every 900, or approximately 0.1 per cent.

Exact data on the frequency of anencephaly in families are not available. A few illustrative cases may be quoted from the literature. Jensen reported multiple births of monsters in a para IX. The first was a 5½ month fetus with spina bifida; then 3 normal pregnancies occurred, followed by a full term infant with spina bifida which lived only 82 days; then a normal pregnancy which was followed by a full term stillborn infant with spina bifida; the eighth was a full term child who had a meningocele and internal hydrocephalus, and the last was a stillborn anencephalus.

Thoms reported 3 anencephalic births in the case of one woman. Anencephaly is rare in twins. Thompson described a case of twins in which one was anencephalic. Still less frequent are conjoint anencephalic monsters. Mudaliar described an anencephalic thoracopagus dibrachius dipus. There is no mention in the literature, however, as far as I could ascertain, of anencephalic separate twins.

REPORT OF CASE

Clinical History: A negress, aged 30 years, para II, gravida III, had labor induced after a diagnosis of polyhydramnios had been made. Two days afterward the membranes ruptured spontaneously, and 5 hours later she was delivered of an anencephalic female monster. Five minutes after the birth a normal placenta was delivered. The membranes and cord were also normal.

The anencephalic monster, delivered by head presentation, weighed 5½ pounds. It began to breathe spontaneously; the pulse rate was 124 and the respiratory rate 30 per minute. One hour and eight minutes after delivery it died and the body was then placed in formaldehyde. Autopsy was not performed for 3 weeks.

AUTOPSY REPORT

The body was that of a full grown anencephalic monster delivered at term. The calvarium was missing and a hemorrhagic soft membrane, known as area cerebrovasculosa, was present, extending from a region just above the eyes to a level corresponding to the occipital protuberance. This meningocele measured 63 mm. transversely, 62 mm. midsagittally and 45 mm. from base to tip. The remainder

of the scalp was covered with hair. The right eye seemed to be well within its socket and measured 17 mm. in width, while the left eye had a narrow upper lid which was proptosed and measured 22 mm. in width (Fig. 1A). The proximal part of the bridge of the nose was missing, being replaced by a membrane which extended from the hemorrhagic membrane which replaced the calvarium. The right nostril was covered by a hump-like soft structure, while the left was almost flat. The width between the outer surfaces of the alae nasi was 25 mm., while that between the inner surfaces was 16 mm. The right nostril was oval, its diameter being one-third of that of the left, which was cleft-like and measured 7 mm. The region of the nasal septum was depressed and continuous with a groove running down a median elongation of the upper lip, which was fused with the lower lip on the left side only (Fig. 1A). It formed the median boundary of a blind oval pouch. Two apertures were thus encountered: on the right a triangular-shaped one (the true mouth) 13 mm. wide, leading into the oral cavity; and on the left a slit-like aperture which led into a blind pouch 12 mm. deep (the false mouth), which had no communication with the oral cavity and was only 3 mm. wide. Thus, there was a complete right and an incomplete left harelip. The ears were grossly normal.

The neck was absent. The chest and abdomen appeared normal anteriorly, but posteriorly (Fig. 1B) there was a depression in the region of the third lumbar vertebra about 28 by 23 mm. in diameter which was covered by a thin dark skin. In the center of the depression was protruding a round soft mass of tissue about 13 mm. in diameter. The external genitalia and extremities appeared normal.

After the soft spongy wall of the meningocele was reflected, fibrous tissue and blood clots were encountered. No trace of cerebrum or cerebellum could be made out. Of the cranial nerves, the right optic and both infra-orbitals were recognized; on microscopic examination I was able to make out the left optic and the seventh and eighth nerves. The medulla oblongata was intact.

A section from the meningocele showed it to be made up of large cells with foamy or vesicular cytoplasm and a small, round or oval eccentric nucleus. A section from the brain site consisted of hemorrhagic connective tissue in which blood vessels, smooth muscle and nerve tissue elements were easily discerned. It was lined with cuboidal epithelium over vascular papillary projections — all re-

sembling the choroid plexus. Nothing that could be identified as hypophysis was found.

The muscles of the right eye were degenerated and so distorted that none could be made out. The eyeball, when cut open, revealed practically no retinal tissue but only a lens with a strip of tissue attached to it. The left eyeball with its muscular attachments was poorly preserved and a section of it showed all eye structures to be well developed except for the iris and ciliary body. The retina was completely detached and thrown into folds — probably an artefact. The optic nerve was normal, though the demarcation between the nerve bundles and the interstitial septa was not plain. There were numerous hemorrhages within the eye tissues but these were most likely due to postmortem changes.

The internal acoustic meatus was 5 by 5 mm. on the left and 4 by 4 mm. on the right. The auricles, external canals, Eustachian tubes and antrums were normal. Microscopic examination of the right ear showed the cavum tympani to be apparently normal. The malleus, incus and one crus of the stapes were intact. The membrana tympani as well as the stapedius and tensor tympani muscles appeared normal. The inner ear showed the vestibule, saccule, utricle and the semicircular canals to be normal. The cochlea was egg-shaped and the modiolus rudimentary. The internal auditory meatus was larger than normal and in the region of the crista transversa there was a deficiency of bone. All the neuroepithelium revealed extensive postmortem changes, precluding a more exact diagnosis.

The left ear showed an apparently normal cavum tympani and drum membrane. The malleus and incus were present. The incostapedial joint was made out; the stapedial head, however, did not join the crura, nor was there a normal footplate. The posterior joint was present as well as the seventh nerve; the eighth, however, was greatly distorted. Both middle ear muscles were present. The stapedius muscle did not join the stapes. The inner ear showed, aside from the three canals, a peculiar short vertical canal anterior to the vestibule and above the level of the cochlea. Its connection could not be determined. The saccule and utricle could not be identified. The vestibule contained in part a very vascular nerve tissue. The round window appeared intact; the cochlea was oval in section and the modiolus was deficient. The internal auditory

meatus was very large and, as on the right side, the bone appeared deficient in the region of the crista transversa.

In brief, the abnormalities consisted of deformities of stapes, abnormal meatus and modiolus on both sides.

Figure 2 shows the two distinct nasal capsules separated by a median sulcus. The right capsule is cartilaginous and wider than the left, which alone is covered by a prolongation of the ossified maxilla. The imperfectly developed asymmetrical orbits appear in Figure 2. The relations of the Eustachian tube were normal, but there was no communication between the nasal cavities and the pharynx. The maxillary and ethmoid sinuses, as well as the larynx, epiglottis and tongue (Figs. 3A and 3B) were normal.

When the head was cut midsagittally it was found that the floor of the left nasal capsule was supported by a mass of tissue which formed the median wall of the "accessory mouth" which is about to be described ("a" in Figs. 3A and 3B). Figure 3A shows the wall intact, while in Figure 3B this space is occupied by the accessory mouth. The length of this mass of tissue, as measured in the center, was 20 mm. from the floor of the nose to the roof of the mouth, as compared to only 5 mm. on the right side. When this median wall was removed a space was encountered in which there was no pharynx or uvula but only a tongue-like bit of tissue 2 mm. long. Histologically it consisted of connective tissue covered with stratified epithelium, without glands or muscle.

The lining of the accessory left mouth had the typical appearance of an oral cavity. The lateral wall of this mouth had six tooth germs, which was also the case on the other side. There were the normal ten in the mandible. As mentioned before, the right mouth was wide with a correspondingly broad maxilla. The tongue was 37 mm. long and the base was 17 mm. thick.

The spinal cord from the medulla to the level of the third lumbar vertebra was normal. The vertebral arches of the third and the fourth lumbar vertebrae were missing; the cord was protruding and came to the surface under the thin membrane (Fig. 1B).

The thymus measured 55 by 30 by 14 mm. In spite of extensive postmortem changes the lobular character of the gland could easily be discerned, as well as the medulla and the cortex with its Hassal's corpuscles.

The thyroid gland, the lower part of the trachea and the main bronchi appeared normal.

The left lung showed a partial fusion of its upper and middle lobes, and the upper lobe revealed superficial markings suggestive of a division to form a third lobe. The right lung had four lobes, the upper lobe being divided by a deep groove running anteroposteriorly to form the fourth. The sectioned lung, aside from great distention of the alveolar spaces, showed no abnormality.

The pericardial cavity, the heart and the great vessels were normal. The abdominal cavity was separated from the thoracic by an abnormal diaphragm whose two halves were not in the same plane. The right half of the diaphragm was lower than the left and the two halves were connected posteriorly by a delicate membrane which formed a pouch extending from the left side into the thoracic cavity on the right, above the right half of the diaphragm and in contact with the posterior surface of the right lung. The upper pole of the spleen, which occupied this pouch, was afforded therefore an intrathoracic position.

The esophagus lay slightly to the right of the midline and was dilated into an imperfectly developed stomach which was partially thoracic in location and lay somewhat to the right of the midline. The "stomach" extended downward in the longitudinal axis of the body, passed anteriorly to the spleen and curved slightly to the left lobe of the liver, where it ended abruptly in a sphincter-like ridge which separated it from the thin-walled duodenum. The latter extended distally almost to the midline, curved slightly to the left and ended in a hardly perceptible duodenojejunal angle. Both the large and small intestines were attached posteriorly by a common mesentery.

The liver and pancreas were normal in gross. Microscopically the architecture of the organs could not be made out because of extensive autolysis.

The adrenals could not be identified in gross or recognized microscopically.

The left kidney consisted of a spongy mass measuring 40 by 16 by 12 mm. The right kidney was 40 by 19 by 16 mm. and like the left appeared honey-combed. Microscopically cellular structure could not be made out because of postmortem changes. Only a bare outline of the cortical and medullary structure could be detected.

In gross the Fallopian tubes and ovaries appeared normal. In sections of the latter there were no Graafian but only primordial follicles.

DISCUSSION

The case reported may be interpreted as one indicating a tendency toward duplication of facial structures. The following series may be recognized.

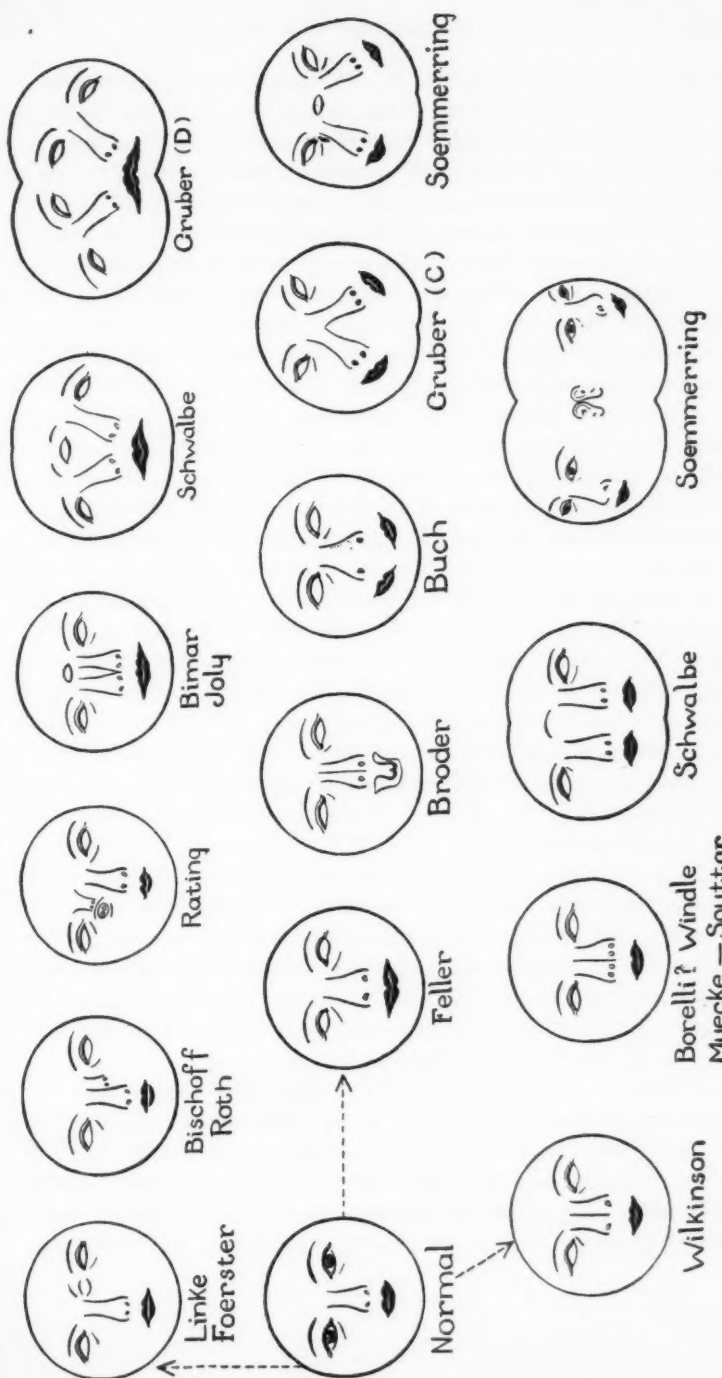
The case of Feller (Text-Fig. 1) showed doubling of the lower jaw with partial doubling of the tongue. My case showed doubling of the lower jaw with partial doubling of the mouth. In Buch's case there was one broad nose and within it a rudimentary second nose; there were two oral cavities and only two eyes, in fact the upper third of the face was normal.

The case of Gruber (Text-Fig. 1) showed two distinct and separate noses and mouths. Soemmerring's case presented a doubling of the oral cavities and nose and the appearance of a third eye.

Förster (Text-Fig. 1) reported a case of doubling of the left side of the frontal bone with the formation of two eye orbits, eyelashes and eyebrows but without a simultaneous doubling of the eyeball. In this mulatto male, 8 months old, who was normal in every respect except for the anomalies of the cranium, there was a small eye cleft with lids and eyebrows but no eyeball on the left side in the usual place, and then somewhat laterad a third larger cleft in which there were a ball, eyelids and eyebrows. On the left side there was a hydrocele. The nose was flat and had only one ala nasi and a blind nostril on the right. The right eye and mouth were normal in gross.

Doubling of the nose is not infrequent. One is dealing here with a broadening of the medially situated nose which, because of an invagination of its medial parts, has led to the formation of a nose called "Doggennase" by Bumba and Lucksch (Text-Fig. 1). The "Doggennase" (congenital malformation in which the nares are divided by a groove) is a result of the failure of the two overlying bones which develop lateral to the cartilaginous nasal septum to unite. As a result of this the septum becomes wider and the nostrils farther apart. Sinking of the septum or its atrophy results in the formation of a double nose which has only one nostril on each side, as in my case.

Rating reported a case (Text-Fig. 1) in which there were something resembling a nose in the region of the right eye, harelip, cleft palate and spina bifida along the entire spinal column. There was a somewhat flat "main" nose in the midline, the right nostril of which



TEXT-FIG. 1. — Modified after a schematic drawing by Bernard Rating in *Virchows Arch. f. path. Anat.*, 1933, 288, 236.

was contracted and the left wide. The accessory nose lay between the root of the nose and the right eye and seemed to have sprung from the orbital roof. Underneath this accessory nose there was, on the right side, a fissure 10 mm. long which was covered with cilia. This contained an eyeball and two lenses, an optic nerve of its own but no cornea; according to the author this was no doubt an anlage for a cyclopic eye. There was, however, no nasolachrymal duct to the accessory nose, which covered the right eye from above. The left eye, in which no tear canals could be made out, was somewhat larger than the right eye, which was 27 mm. from the midline, while the left eye was only 10 mm. from it.

According to Hübner, "all gradations of a diprosopia ranging from a doubled hypophysis through a 'Rhinodymie' and ending with a diprosopus sensu strictiori are possible." In the latter case the skull is incomplete, the face incompletely or completely doubled and this doubling may range from "Rhinodymie" to di-cephalus.

Lasagna reported a case of double nose in a child, 2 months old, with no other abnormalities. The left nose had a small aperture, while the right was normal in every way. The pinhead-sized opening referred to and interpreted by the author as a rudimentary "tear collector" was in the left infra-orbital region.

Bischoff and Roth (Text-Fig. 1) have described a case in which one nose was in the normal midline position while the other had its origin from above the left orbit. There were four frontal bones and the mouth was normal.

The case of Bimar, a typical "Rhinodymie," was first recognized in 1881, and since then some 6 cases have been described.

A further step toward duplication of the face is represented by the case of Schwalbe in which there were two mouths, two noses and three eyes (a true "Rhinodymie"). Finally, the last stage is exemplified by Gruber's case (last drawing in the first row) in which there were four eyes, two noses and two mouths.

Wilkinson (Text-Fig. 1) described a deep depression in the midline of the nose, wide separation of the nostrils, and broadening of the whole feature. On the inner side of each vestibule the anterior ends of the nasal septum could be seen as a proximal ridge. The two sides of the septum having been separated from one another, there was no nasal obstruction. He pointed out that failure of the median

walls of the nasal cartilage to fuse is rare, and that such a failure might account for a median cleft palate and a bifid nose.

A further stage toward duplication is presented in the cases of Windle, and Muecke and Souttar. Windle (Text-Fig. 1) reported the case of a girl, aged 5 years, who had a nose that was incompletely divided into two parts by a longitudinal furrow. There were four nostrils, two on each side of the septum, and two more laterad. The two median ones were functionless, small, blind and only 10 mm. deep. The normal, wider pair functioned properly. The only other malformations were two ridges on the upper lip. The author believed that this represented an attempt at division of the nose into two noses.

Muecke and Souttar, who reported a case of two completely formed noses in a girl, aged 3 years, the right being more central and larger than the left, but each having its own septum, nostrils and well formed alae nasi, postulated that the nature of such an anomaly depends on whether or not the two anlage are present. When they are present the medial depression is not flat but is deeply grooved, so that two equally well developed noses lie one next to the other, each with its individual septum, alae and nostrils. Finally, there are stages of symmetrical doubling represented by the cases of Schwalbe, and especially that of Soemmerring (Text-Fig. 1).

Thus, there may occur all gradations of doubling from incomplete to that of diprosop, tetraphthalmic, tetrorbitus, diotus, monosomus, dignathus and monauchenos.

In summary, it may be said that monsters of this type may be divided into three series, as shown in Text-Figure 1. The tendency in the first row is toward duplication of nose and eyes, eventually reaching the mouth. In the second row the mouth and nose are involved at first with a tendency to include the eyes. In the third row all the elements are duplicated.

SUMMARY AND CONCLUSIONS

1. The case reported belongs in the group of "Rhinodymie," as described by Rating, and forms a link between the case of Feller, showing doubling of the lower jaw only, and that of Buch with two well developed and distinct mouths and only a rudimentary second nose (Text-Fig. 1).

2. The anatomical findings and the consideration of cases described in the literature suggest that doubling of the nose and mouth are expressions of a tendency toward dicephaly. These were associated in the case reported with anencephaly, deformities of stapes, meatus and modiolus on both sides, defect of the diaphragm, imperfectly developed stomach, absent adrenals and spina bifida.

3. Experimental evidence, which alone is significant, indicates that anencephaly is due to an inhibition in the early development of the embryo affecting regions of highest rate of development. The frequent association of anencephaly with cephalic duplications suggests that the latter may be due to similar factors.

4. The deficient diaphragm may be interpreted as an interference with the development of the pleuroperitoneal membrane.

5. Much of the speculation concerning the causes underlying human monsters is futile. The only direct evidence bearing on the subject at the present time is that of experimental embryology.

NOTE: This paper was inspired by Dr. George W. Bartelmez, Professor of Anatomy, University of Chicago. I also wish to acknowledge my indebtedness to Dr. Robert S. Jason, Assistant Professor of Pathology, Howard University School of Medicine, for the study of microscopic sections of the viscera; Dr. Elmer W. Hagens, Assistant Clinical Professor of Laryngology and Otolaryngology, Rush Medical College, for the study of the sections of the ears; and to Dr. Max L. Folk, Assistant Professor of Ophthalmology, College of Medicine, University of Illinois, for the study of the eye sections.

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DESCRIPTION OF PLATES

PLATE 110

- FIG. 1. Anencephalic fetus.
 A = ventral view.
 B = dorsal view.



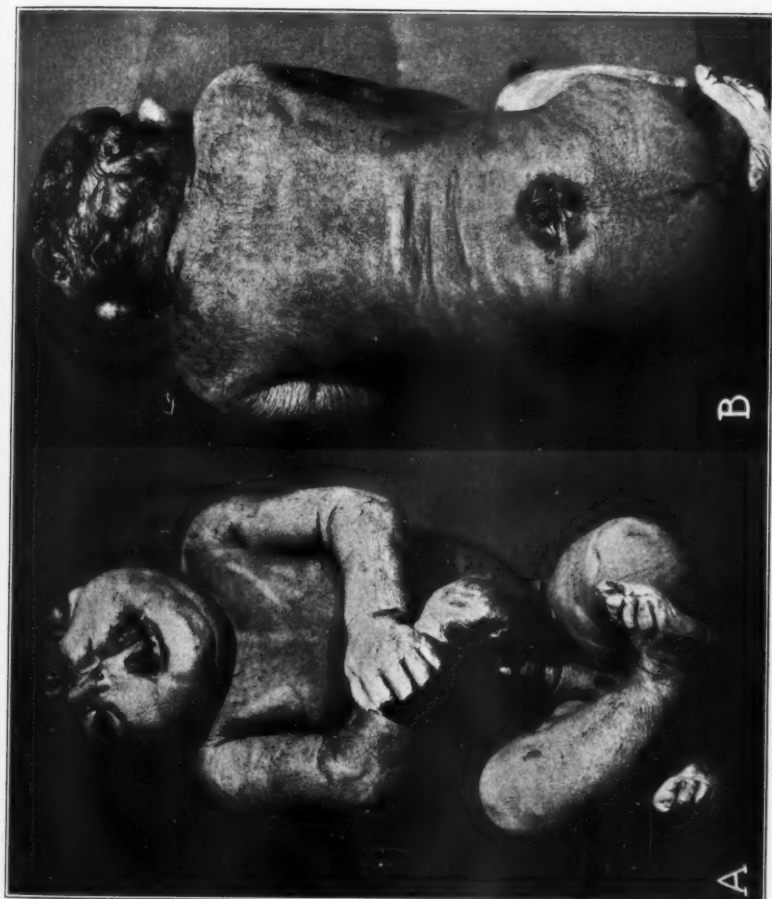


PLATE III

FIG. 2. View from above of the inner base of skull.

- a = free margin of squama occipitalis.
- b = free margin of temporal bone.
- c = internal acoustic meatus.
- d = right cartilaginous nasal capsule.
- e = maxillary region of skull.
- f = left eyeball.
- g = right optic foramen.
- h = medulla oblongata in foramen magnum.

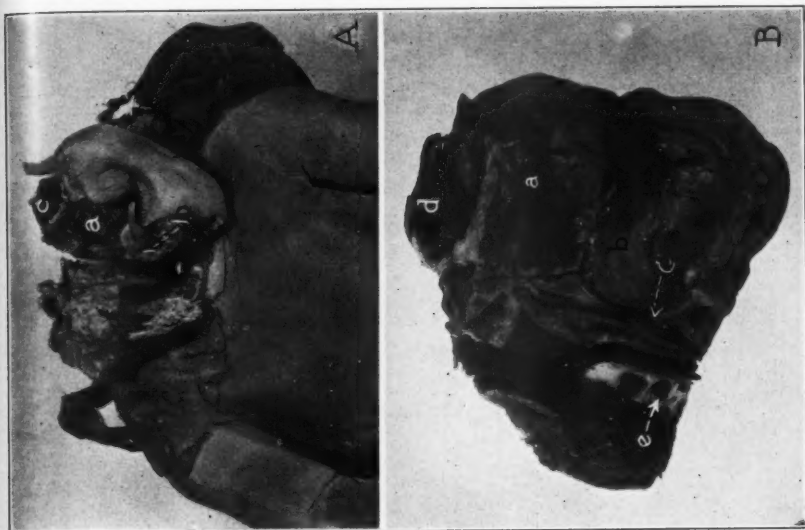
FIG. 3A. Two halves of the head separated to show the accessory mouth.

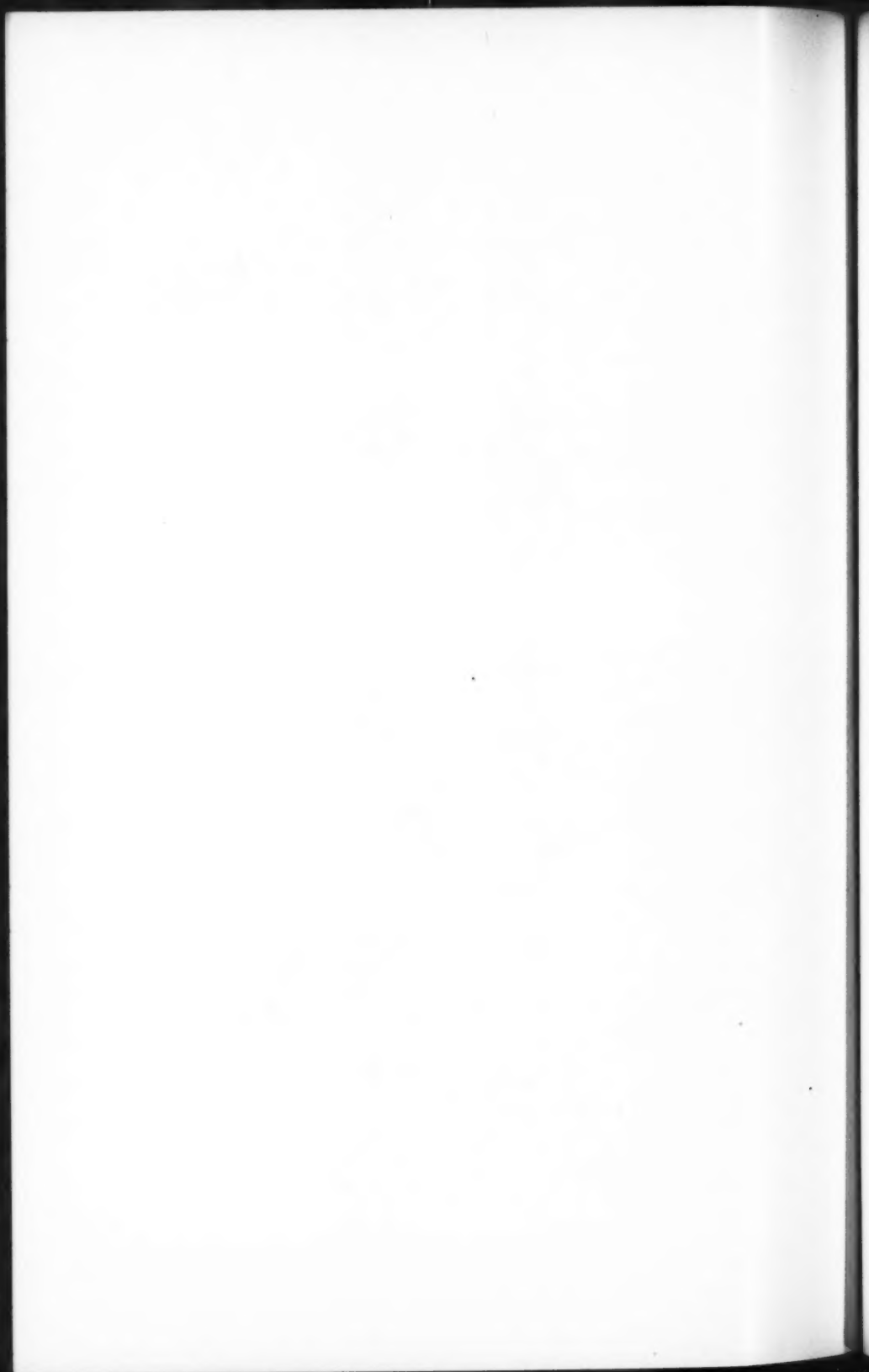
- a = median wall of the accessory mouth underneath the left maxilla.
- b = base of tongue.
- c = left turbinates.

FIG. 3B. View from above of the medial side of the left moiety.

- a = space occupied by the accessory mouth.
- b = tongue in true mouth.
- c = epiglottis.
- d = left turbinates.
- e = ossified centrum of the third cervical vertebrae.







ANOMALIES OF THE CIRCLE OF WILLIS WITH RESULTING ENCEPHALOMALACIA AND CEREBRAL HEMORRHAGE *

OTTO SAPHIR, M.D.

*(From the Department of Pathology of the Nelson Morris Institute of Medical Research,
Michael Reese Hospital, Chicago, Ill.)*

INTRODUCTION

There is an increasing tendency today to attribute to functional abnormalities the anatomical changes that are undoubtedly the result of vascular lesions. This pertains particularly to encephalomalacia and cerebral hemorrhage. Fischer-Wasels¹ stated very recently that in instances of brain hemorrhage there is primarily a destruction of brain tissue as a result either of trauma or of toxins, or because of local circulatory disturbances. As a result of the primary destruction split products may be present which secondarily damage the walls of blood vessels, either by accentuating the local disturbances of circulation or by producing anatomical changes in the vessels which finally may result in necrosis. The damage to the walls of the vessels may also cause reflex disturbances. Weil,² realizing the difficulty in evaluating the different theories of explanation of cerebral hemorrhages and encephalomalacia, stated that different possibilities may exist, primary vascular disease or primary functional disturbances of vasomotor regulation. Although it cannot be denied that apparent vascular lesions may very occasionally be the result of functional disorders which cannot be demonstrated by means available to the morphologist, it cannot be emphasized sufficiently that every possible morphologically demonstrable cause of vascular lesions must be searched for, carefully evaluated and ruled out before the morphologist resorts to an explanation based on functional disorders. The assumption of functional disorders is often a confession by the morphologist of inability to find the real cause of the lesion in question.

In the following, 2 cases of encephalomalacia and cerebral hemorrhage will be reported. In both of these, vascular disturbances were not the result of an occlusion of the vessels, and the causes of these

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lesions were at first difficult to determine. Only a careful examination of the vessels revealed anomalies of the circle of Willis as the underlying cause of the brain abnormalities. Also, a third case of anomalies of the circle of Willis not accompanied by vascular disturbances of the brain will be reported.

The literature on this subject is very scant. It may be divided into three parts — one dealing with the onto- and phylogenetic development, one statistical, and one associating the anomalies with certain psychic features. It may be of interest to note that anatomical brain lesions, as far as could be ascertained, were not correlated with anomalies of the circle of Willis. There are, however, references to cerebral aneurysms associated with anomalies of the arteries of the circle of Willis, and the opinion is expressed that abnormalities in the distribution of the blood because of such anomalies may be a mechanical factor in the causation of cerebral aneurysms (Jacques³). Only the more important references pertinent to this communication and principally confined to the posterior communicating branches of the circle of Willis are given.

LITERATURE *

Webber⁵ in 1882 described a case of an abnormal distribution of the circle of Willis. The right posterior communicating artery was one-tenth its usual diameter and the right posterior cerebral was twice its usual size. The left posterior communicating artery was nearly twice its normal size and was virtually the origin of the left posterior cerebral artery.

Windle⁶ in 1888 examined 200 brains and found both posterior communicating arteries extremely small in seven instances. Both were absent in three. He stated that the origin of the posterior cerebral artery from one or both internal carotid arteries was the most common variety.

De Vriese⁷ in 1905 stated that in fish, amphibians, reptiles, birds and some mammals the internal carotid artery provides the only arterial supply to the cerebrum. Upon entering the cranial cavity each internal carotid divides into two branches, the cranial and the caudal. The terminal branches of the caudals unite to form the

* The earlier literature is given in Mitchell's⁴ article.

basilar artery. In mammals the circle of Willis is formed either by branches of the internal carotid arteries (so-called "primitive type") or is formed by branches of the vertebral arteries (so-called "recent type"). The following anomalies of the posterior communicating arteries were found.

- (1) The caliber of these vessels may be larger than normal.
- (2) Variations in the caliber of the right and left arteries.
- (3) Complete absence of one or both vessels.
- (4) One or both vessels may be so large that the posterior cerebral artery appears to be a terminal branch of the posterior communicating. The divisional branch of the basilar artery up to the point of union with the posterior communicating branch (most proximal portion of the posterior cerebral) may be so small that it appears to be the posterior communicating artery.
- (5) The basilar artery may be formed by the fusion of the posterior communicating and the divisional branch of the basilar artery. (The posterior communicating artery and the divisional branch of the basilar artery are equal in size.)

Blackburn⁸ in 1907 stated that an enlargement of one or both posterior communicating arteries was a common anomaly. In almost every specimen enlargement of the posterior communicating arteries coincided with small posterior trunks at their point of origin from the basilar or at their proximal portions.

Stopford⁹ stated in 1916 that in 105 brains out of 111 there was a complete anastomosis of the circle of Willis. In 6 of the remaining brains the circle of Willis was incomplete because of the absence of the posterior communicating branch on one or both sides.

Berger¹⁰ in 1923 described the transformation of the left posterior communicating artery into a fibrous cord. The right posterior communicating artery was very large. The right posterior cerebral artery seemed to be a continuation of the right posterior communicating artery.

Walcker¹¹ in 1924 stated that there are two types of circle of Willis. One is characterized by its patency, the other by an interruption of its continuity. The former is referred to as "closed" circle, the latter as "open" circle. The closed circle of Willis is found frequently in individuals with a mesaticephalic and a slightly brachycephalic configuration of the head. In the decidedly brachycephalic there often is an increased number of anastomoses seen in

the circle of Willis. The open circle of Willis is often present in individuals with dolichocephalic configuration of the head.

Shellshear¹² in 1926 described the arteries of the brain of an orangutan and stated that the arteries of the brain are stable phylogenetically and ontogenetically.

Jacques³ in 1926 found a circle of Willis abnormally incomplete. The right posterior communicating artery took the course normally taken by the right posterior cerebral artery. At the site of origin of the left posterior communicating artery was an aneurysm measuring 2.5 cm. in greatest diameter.

Voris¹³ in 1928 described the arterial supply of the brain in the Virginian opossum (*Didelphis virginiana*) and found a double communication between the internal carotid and the basilar artery. One was formed by the posterior communicating and the second one by a small branch of the medial cerebral artery which came off immediately after its origin. There was no anterior communicating artery.

Hindze and Fedotowa¹⁴ in 1931 described a case of absence of the posterior communicating artery on one side. This vessel on the other side was very large and seemed to be the origin of the posterior cerebral artery. These authors described seven types of anomalies as follows:

(1) *Primitive Type*: The posterior cerebral artery forms the continuation of the internal carotid artery. The posterior communicating artery is larger than normal.

(2) *Transitional Type*: The posterior cerebral arteries are formed about evenly by the posterior communicating and the divisional branches of the basilar artery.

(3) *Recent Type*: The posterior cerebral artery is formed principally by the branches of the basilar artery.

(4) The internal carotid and basilar arteries are united by an embryonal trigeminal artery, or (5) hypoglossal artery.

(6) *Mixed Type*: On one side the posterior communicating artery is absent and on the other side it is much larger than normal.

(7) Complete separation of the circulation of the internal carotid and vertebral arteries.

De Vriese⁷ found anomalies of the circle of Willis for the most part in criminals and in the insane.

Blackburn⁸ pointed out that in only 65 out of 220 brains of consecutive cases of mental disease was the circle of Willis normal.

Hindze¹⁵ found anomalies of the circle of Willis in a schizophrenic, in a criminal, and also in a number of outstanding individuals — scientists and poets.

Wyubow¹⁶ stated that anomalies of the anterior cerebral arteries were found in 22.3 per cent of the insane and in criminals.

Parnizetti¹⁷ found anomalies of the circle of Willis in 55.1 per cent of criminals examined.

CASE REPORTS

CASE 1. Clinical History: A 50 year old female was treated intermittently with iodine over a period of 6 years for a toxic goiter. Her complaint on admission to the hospital was shortness of breath, palpitation and swelling of the legs. Physical examination revealed an undernourished patient. The heart was enlarged and there was a systolic murmur at the apex. The liver and spleen also were enlarged. She had an anxious expression and a stare, but no exophthalmus. The right lobe of the thyroid was enlarged. The basal metabolic rate was + 79. She was given iodine and her basal metabolic rate dropped to + 49 in 3 days. Digitalis was administered and surgical removal of the thyroid was contemplated but not performed. Seventeen days after admission her temperature rose to 103.6° F. The white count at that time was 6900. The temperature dropped to normal but occasionally rose again. She developed marked psychic symptoms such as incoherent talking and crying, and became irrational. Later the temperature rose to 106.4° F. and she died of what was considered to be thyroid crisis.

Autopsy Report

At autopsy there was a recent and organizing bronchopneumonia and a fatty infiltration of the heart involving mainly the right ventricle. There was a coronary arteriosclerosis and myocardial fibrosis, dilatation of the heart, chronic passive hyperemia of lungs, liver, spleen and kidneys and edema of the lower extremities. A nodular colloid goiter was found which histologically revealed foci of hypertrophy and hyperplasia. The vessels of the base of the brain showed the following abnormalities. The left posterior cerebral artery took its origin from the left internal carotid artery by means of a large posterior communicating artery. There was a fibrous cord without any recognizable lumen running between the first portion of the left posterior cerebral (or proximal portion of the posterior communicating) and the end portion of the basilar artery where the posterior cerebral artery starts normally. The right posterior cerebral artery

took its origin from the basilar artery. There was no communication between the right internal carotid and the right posterior cerebral artery. The anterior cerebral artery and the anterior communicating branch were normal. A moderate degree of arteriosclerotic change was found throughout the arteries of the base of the brain. Multiple sections of the brain revealed two areas of encephalomalacia which were rather recent and which were found in the white substance of the cerebrum close to the right lenticulate nucleus. Small areas of hemorrhage were also found in the vicinity of the encephalomalacia.

Histological examination of the brain revealed, in addition to encephalomalacia and hemorrhage, various degenerative changes of the ganglion cells. Many of the nuclei were absent, the cytoplasm of these cells was not well defined, was pale, and in many fields only shadows of ganglion cells could be made out.

Summary

A 50 year old female developed mental symptoms, became irrational and died of what was considered to be a thyroid crisis. At autopsy, in addition to other pathological findings, encephalomalacia and cerebral hemorrhages were found. There was a complete interruption of the circle of Willis and no communication between the internal carotid and the vertebral arteries. There was an arteriosclerosis of the vessels of the base of the brain.

CASE 2. Clinical History: This patient was an adult white male, 70 years of age, whose blood pressure had been elevated for a number of years. For the past 10 years he had experienced attacks of complete paralysis affecting the extremities and face, with loss of consciousness. These attacks lasted about 3 or 4 days; occasionally, however, only a few moments. More commonly the left side of the body was involved. Four months before death he had an attack of precordial pain which was interpreted as coronary thrombosis. His mental condition most of the time was good, although in the last few months preceding death there had been periods of mental disturbance. The arterial blood pressure was constantly around 220/150. During the last few weeks of life he had hematuria. Bronchopneumonia developed and he died shortly afterward.

Autopsy Report

At autopsy there was a generalized arteriosclerosis with marked involvement of the coronary arteries and occlusion of several branches. There were old and recent infarcts in the myocardium of

the left ventricle, dilatation of the heart, chronic passive hyperemia of lungs, liver, spleen and kidneys, and edema of the lower extremities. A nephrosclerosis of the arteriolar variety, hypertrophy of the heart and a bilateral confluent bronchopneumonia were also found. There was a carcinoma of the urinary bladder. The arteries of the base of the brain revealed the following abnormalities: The right posterior communicating branch of the circle of Willis was absent and the left posterior communicating branch was transformed into a very thin fibrous cord. In the anterior-superior portion of the left frontal lobe near the longitudinal fissure there was an area of hemorrhage measuring 0.5 cm. in diameter. This lesion was located partially in the gray and partially in the white matter. An area of softening was observed anteriorly to the anterior horn of the left ventricle close to its wall. An area of old encephalomalacia was seen in the right anterior portion of the corpus callosum. The surrounding white substance showed small, yellowish-tinged cystic cavities. Close to the left internal capsule another area of encephalomalacia was found which measured 1 cm. in diameter. The left parietal lobe revealed a deep red area of hemorrhage within the white matter extending over an area 0.5 cm. in diameter. Frontal sections through the right occipital lobe revealed a soft, yellow, necrotic area which measured 1 by 2 cm. in diameter. A small red area of hemorrhage was also found in the gray matter of the midportion of the occipital lobe.

Histological examination of the brain revealed areas of recent and old encephalomalacia and hemorrhage. Small cysts were seen which were lined with phagocytic cells containing many yellowish brown pigment granules. Other fields showed many scavenger cells and only indistinct outlines of brain tissue. The cytoplasm of many ganglion cells was pale and often vacuolated. The glia nuclei were preserved.

Summary

The brain of a 70 year old man whose main clinical symptoms were attacks of unconsciousness revealed an interruption of the circle of Willis and resulting separation of the circulation of the internal carotid and vertebral arteries. There was a marked arteriosclerosis of the arteries at the base of the brain and multiple areas of encephalomalacia and hemorrhage into the brain.

CASE 3. *Clinical History:* A 65 year old male complained of rapidly increasing shortness of breath, nocturnal cough, and swelling of the ankles. On physical examination marked cyanosis was present and the cardiac dullness was enlarged. There was a loud systolic murmur over the entire precordium. The patient was mentally confused during the last 10 days of his life. The clinical impression was a generalized arteriosclerosis with coronary arteriosclerosis and myocardial fibrosis.

Autopsy Report

The autopsy revealed a marked generalized arteriosclerosis with involvement of the aortic valve and stenosis of its orifice. There was a coronary arteriosclerosis, myocardial fibrosis, hypertrophy and dilatation of the heart and generalized chronic passive hyperemia. There also was a bilateral bronchopneumonia. The circle of Willis showed the following changes: Both posterior communicating branches were much thinner than normal. Their lumens were not recognized. No openings were found at the usual sites of origin of both internal carotid and both posterior cerebral arteries. Multiple sections of the brain showed no gross changes.

The histological examination revealed marked degenerative changes of ganglion cells with necrosis in some instances. There were no areas of encephalomalacia or hemorrhages.

Summary

The brain of a 65 year old man who was mentally confused during the last 10 days of his life and who died of myocardial failure complicated by bronchopneumonia revealed a marked hypoplasia of both posterior communicating arteries. These vessels were reduced to the extent of prohibiting circulation of blood through them.

DISCUSSION

The first two brains are interesting in that both showed areas of encephalomalacia and cerebral hemorrhages in the absence of occluding lesions of the cerebral arteries. In each case there was a severe arteriosclerosis of the arteries of the brain and in each anomalies of the circle of Willis which principally involved the posterior communicating arteries could be demonstrated.

Stopford⁹ stressed the point that the posterior communicating arteries were of much greater importance during the early weeks of intra-uterine life than in the later periods. This is so because in the

early period they form the origin of the posterior cerebral artery from the internal carotid arteries. Later, when the posterior cerebral artery is reinforced by anastomosis with the basilar artery, the posterior communicating artery is no longer essential for the maintenance of the blood supply. Yet under normal conditions the channel between the internal carotid and basilar arteries remains open and can be used as a collateral vessel for either the internal carotid or basilar arteries and their branches. How frequently this channel is used in this compensatory fashion, and its importance, cannot be estimated. As the literature shows, and as was brought out by the 3 cases, the posterior communicating branches are not essential in the maintenance of the circulation of the brain under normal conditions. Since particular attention was paid to the posterior communicating arteries, changes in the caliber of these vessels were found quite frequently. Most commonly they were very thin. In instances of diffuse arteriosclerosis of the vessels of the base of the brain, however, the free unhampered collateral anastomosis of the circulation of the internal carotid and vertebral arteries seems essential, particularly in view of the fact that the cerebral arteries are end-arteries. In other words, an interference with the passage of blood through the circle of Willis does not make itself manifest until the circulation through the internal carotid and vertebral arteries is impaired. In the first 2 cases two causes for such impaired circulation are demonstrable, namely the arteriosclerotic plaques throughout the arteries of the brain, and the failing heart, evidence of which could be deduced from the findings of myocardial fibrosis, chronic passive hyperemia of the various organs, and edema of the lower extremities and the dilated heart. As a result of the impaired *vis a tergo* and of the arteriosclerosis of the vessels of the base of the brain, complicated by the complete separation of the two arterial channels of the brain, encephalomalacia and hemorrhages into the brain ensued. This conception of the cause of the brain lesion is based entirely on morphologically demonstrable lesions, not only of the brain itself but also of the various organs, particularly the heart. It may be mentioned in this connection that Fleming and Naffziger¹⁸ called attention to the danger of vascular disturbances of the brain in instances of a fall of the arterial pressure.

As was previously emphasized, the anastomosis between one posterior cerebral artery and the basilar artery was insufficient in the

first case. Stopford⁹ pointed out that under these circumstances, as was also seen in this brain, the posterior cerebral artery appears to be a branch of the internal carotid artery. He mentioned that what appears to be a compensatory enlargement of the posterior communicating artery to accommodate for its abnormally small origin from the basilar artery is strictly speaking a persistence of the embryonic condition.

Very interesting is Case 2. Clinically the many attacks of unconsciousness followed by paralysis of the extremities could not definitely be explained. At autopsy a number of areas of encephalomalacia and hemorrhage were found. Whereas it would have been difficult to explain the multiplicity of these lesions on anatomical grounds in the absence of multiple emboli or thrombi, the anomaly of the circle of Willis combined with the arteriosclerosis and anatomical evidence of failing heart, makes an explanation on morphological grounds possible. Of course it is impossible to state whether or not in addition to morphologically demonstrable lesions, physiopathological, merely functional conditions played an additional rôle in the causation of the infarcts. But, as a matter of principle, such functional causes should be taken into consideration only when a careful examination fails to yield an explanation based on morphological grounds.

In Case 3 the vessel anomaly and degenerative changes in the brain were the only significant findings. It is conceivable that the degenerative changes were the result of beginning deprivation of arterial blood supply. Similar degenerative lesions were also noted in the first 2 cases. It is of interest that all three patients were mentally confused during the last week of life. This confusion may have coincided with the beginning of the final myocardial failure.

As stated in the literature, anomalies of the circle of Willis have been correlated with psychic disturbances, since these anomalies have been found in a number of insane individuals and in criminals. On the other hand, such changes were also present in the mentally alert (Hindze¹⁵). The three patients who were the subject of this communication were mentally normal as far as could be determined by the investigation of the clinical records and by subsequent inquiries among relatives.

SUMMARY AND CONCLUSIONS

Anomalies of the circle of Willis, with resulting interruption of the circulation between the internal carotid and vertebral arteries, may form the anatomical basis of cerebral vascular disturbances. The recognition of such anomalies is significant because they aid in the explanation of cerebral hemorrhage and encephalomalacia on morphologically demonstrable grounds in the absence of occluding lesions of the supplying arteries.

In addition to local causes for encephalomalacia and cerebral hemorrhage one must consider also the condition of the myocardium and evidence of myocardial failure in the various organs.

Three brains are described which revealed anomalies of the circle of Willis involving the posterior communicating arteries, and an abnormal origin of the posterior cerebral artery in 1 case. Two of these brains revealed areas of encephalomalacia and cerebral hemorrhage, without the presence of occluding lesions in the supplying arteries. Whereas the posterior communicating arteries are not essential in the maintenance of the circulation of the brain under normal conditions, a free unhampered collateral anastomosis between the internal carotid and vertebral arteries is important in instances of diffuse arteriosclerosis of the arteries of the base of the brain combined with beginning myocardial failure.

This conception of the origin of these brain lesions is based entirely on morphologically demonstrable changes and does not require the assumption of theoretical functional disturbances of the circulation. Perhaps similar anatomical findings may explain anatomical changes elsewhere which now are attributed to functional disturbances.

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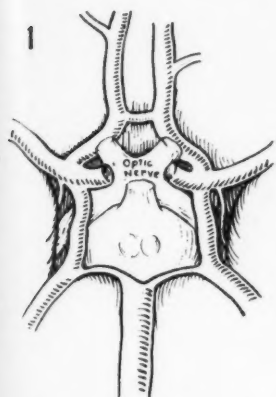
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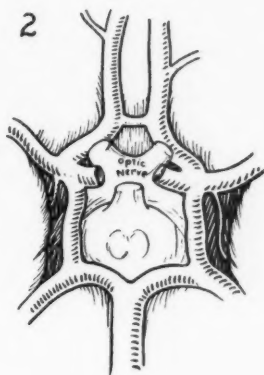
DESCRIPTION OF PLATE

PLATE 112

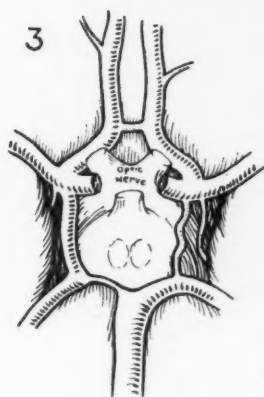
- FIG. 1. So-called *primitive type* of the circle of Willis. The posterior cerebral artery forms the continuation of the internal carotid artery. The posterior communicating artery is larger than normal. The branches of the divisional basilar artery are small.
- FIG. 2. So-called *transitional type* of the circle of Willis. The posterior cerebral arteries are formed by the posterior communicating and the divisional branches of the basilar artery. These two branches are of about equal size.
- FIG. 3. So-called *mixed type* of the circle of Willis. The left posterior communicating branch is normal (*recent type*) and the right posterior communicating artery corresponds to the *primitive type*.
- FIG. 4. The normal circle of Willis and the anomalies found in the first case are given for comparison. The left posterior cerebral artery takes its origin from the left internal carotid by means of a large proximal portion of the posterior communicating artery. The distal portion of the communicating branch and the divisional branch of the left basilar artery are transformed into a fibrous cord. The right posterior communicating artery is absent.
- FIG. 5. Case 2. The left posterior communicating artery is transformed into a fibrous cord. The right is absent.
- FIG. 6. Case 3. Both posterior communicating arteries are transformed into a fibrous cord.



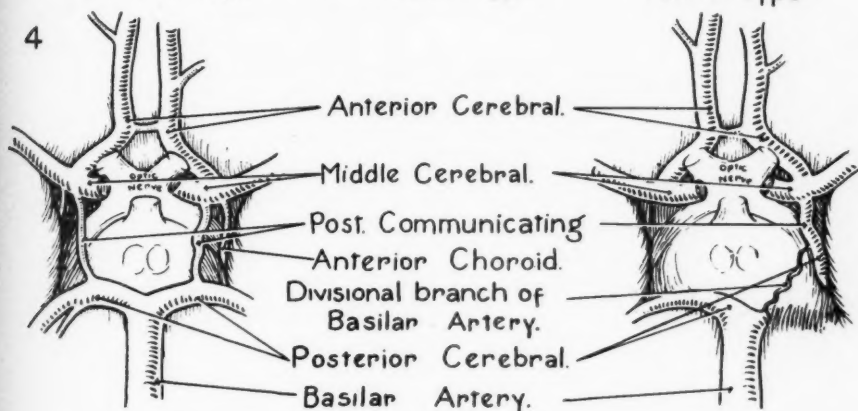
Primitive Type



Transitional Type.

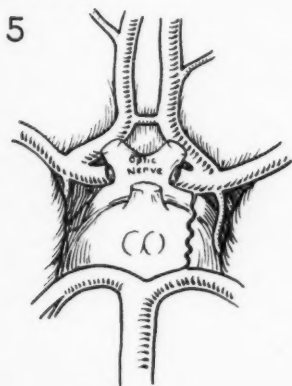


Mixed Type

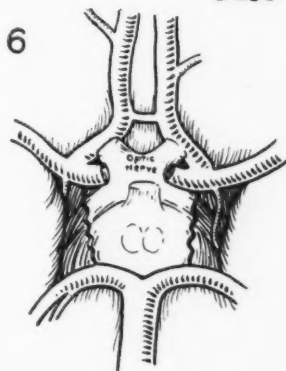


Normal

Case 1.



Case 2.



Case 3



EXPERIMENTAL GASTRIC EROSIONS FOLLOWING HYPOTHALAMIC LESIONS IN MONKEYS *

E. C. HOFF, Ph.D., AND D. SHEEHAN,† M.D.

(From the Laboratory of Physiology, Yale University School of Medicine,
New Haven, Conn.)

INTRODUCTION

Cushing,¹ in his Balfour Lecture, has directed attention to some neurogenic factors involved in acute ulceration of the gastro-intestinal tract. In a comprehensive review of the literature on the subject he points out that the weight of evidence indicates a causal relation between hypothalamic disturbance and certain gastro-intestinal lesions.

Although it had long been suspected that the central nervous system plays a part in the genesis of gastro-intestinal ulceration (Schiff,² and Brown-Séquard³), the experiments of two Russian investigators, Burdenko and Mogilnitzki,⁴ were among the first to provide direct evidence that hypothalamic injury might lead to haemorrhagic ulceration in the stomach and duodenum. Using a subtemporal approach they produced small lesions in the base of the brain immediately behind the infundibular stalk. Gastric haemorrhages and erosions, acute ulceration and occasionally perforation with peritonitis resulted and in some instances, in animals surviving the operation by several months, chronic cicatricial ulceration was found. They explained their results on the basis of a destruction of a vasomotor centre in the posterior hypothalamus and of a metabolic centre in the tuberal region.

Keller, Hare and d'Amour,⁵ in a long series of experiments on cats and dogs in which many and varied types of experimental lesions had been made in the upper brain stem, found that apart from 3 chronic midbrain animals in which erosions occurred in the stomach acute gastro-intestinal changes most commonly followed lesions that had been associated with haemorrhage into the cerebral ventricles, *i.e.* after a transverse section of the brain at the level of the chiasma.

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† Rockefeller Fellow.

Multiple erosions of the stomach were encountered in some of the cases after hypothalamic injury. They occurred most frequently in the body of the stomach, rarely in the pylorus, and were most numerous on the crests of the folds.

Watts and Fulton⁶ have recently reported a series of experiments in monkeys in which localised hypothalamic injury was associated with acute gastro-intestinal changes. Of 17 animals with large hypothalamic lesions 4 developed gastric erosions, 1 died of a perforated duodenal ulcer and several showed mucosal haemorrhage. A careful examination of the gastro-intestinal tract in a control series of 63 animals revealed that gastric erosions were found in only 1 monkey without a hypothalamic injury, and this animal had received daily injections of ephedrin after a midthoracic transection of the spinal cord. Their observations did not permit any conclusions as to the relation between the destruction of any single group of hypothalamic nuclei and the pathological changes in the gastro-intestinal tract. They were inclined to the view that the erosions following lesions in the tuberal and supra-optic regions were due primarily to local ischaemia incident to hyperactivity of the sympathetic vasoconstrictor mechanism of the gut.

As the observations of Watts and Fulton⁶ were based on relatively long term experiments, they suggested that further studies were needed with earlier sacrifice; the present experiments have been undertaken with this object specifically in view.

METHOD

This report is based on a study of 19 monkeys (11 capuchins, *Cebus fatuellus*, and 8 rhesus, *Macaca mulatta*) in each of which a small lesion was made in the hypothalamus. In order to provide an adequate control series a careful examination was made of the gastro-intestinal tract of all primates sacrificed in this laboratory for a period of 6 months. These (some 50 in all) had been subjected to various operative procedures, including occlusion of the pituitary stalk, spinal cord transection and ablation of certain cortical areas, frontal, premotor and occipital.

Operative Procedure

A subtemporal approach was used throughout this investigation. This method was selected in order to avoid damage to any part of the central nervous system, other than the hypothalamic region, as the animals were also being used for anatomical studies of nerve pathways. All operative procedures were carried out with full aseptic precautions and under sodium amytal anaesthesia.

A large unilateral bone flap extending across the midline was turned down in each case. With the head of the animal placed on its side the temporal lobe was gently retracted upwards from the base of the skull, thereby exposing the optic and oculomotor nerves and the infundibular stalk partially screened by the internal carotid artery. By means of a sharp hooked probe a small lesion was made in a selected part of the hypothalamus, either directly in the tuber cinereum or in the posterior hypothalamic region. An attempt was made to produce a bilateral lesion in every case. Little haemorrhage was encountered, though frequently a small amount of cerebrospinal fluid escaped from the puncture. The temporal lobe was allowed to slip back into position, the bone flap replaced and the wound closed.

During the elevation of the temporal lobe there was often a marked slowing of the heart rate. In the earlier experiments, in order to combat this effect, a small dose (0.3 to 0.5 cc.) of adrenalin was administered subcutaneously at this stage of the procedure, but this was dispensed with in later experiments.

Postoperative Study

Most of the animals survived 3 days and were sacrificed at the end of this period as it was particularly desirable in this investigation to observe the early appearances of any gastric lesions. Two of the animals were sacrificed at the end of 3 weeks and were utilised for Marchi studies. During the postoperative period careful observations were made of the general condition of the animal and the faeces and any vomitus were examined for blood.

At the time of sacrifice the animals were profoundly anaesthetised with ether. The stomach and intestines were isolated from the general circulation, removed and placed in physiological saline for immediate examination. The animal was then injected through the

abdominal aorta with 500 to 1000 cc. of 0.9 per cent saline until clear fluid flowed from the opened inferior vena cava. This was followed by 300 to 500 cc. 10 per cent chloral hydrate solution. The brain and spinal cord were removed at once and a complete autopsy performed.

Histological Technique

The hypothalamus and adjacent brain tissue were removed in one piece and placed in 75 per cent alcohol. Serial Nissl-stained sections were made of the entire block so that the precise localisation of the lesion could be ascertained in each case. The rest of the brain stem and sample blocks from the spinal cord were stained with Cajal's method of reduced silver nitrate impregnation (formula 6a) and serial sections made for examination of degenerated *bouton terminaux* (as described by Hoff ⁷). Any lesions found in the gastrointestinal tract were excised and fixed in neutral formalin solution and sections were stained with haemalum and eosin.

RESULTS

Postoperative Course

The animals showed striking individual variations in the general postoperative condition. Some made a rapid recovery so that within 24 hours their behaviour was apparently entirely normal. Many of the animals, on the other hand, were profoundly affected by the operation. They recovered slowly from the anaesthesia and sat hunched up in the cage with the head lowered between the upper limbs. These animals refused food and invariably their condition became progressively worse, death supervening in 1 to 3 days.

Subsequent histological examination of the base of the brain revealed nothing in the location or extent of the lesion that would account for the marked difference in postoperative recovery. Thus, in Experiment 7, in which serial sections of the hypothalamus showed a bilateral lesion that had destroyed the tuber cinereum, opened up the base of the third ventricle and had all but severed the infundibular stalk, the animal was active and feeding several hours after completion of the operation. It continued to improve so that on the following morning its general behaviour and activity were indistinguishable from those of an unoperated animal. Sacrifice 3

days after the operation revealed no abnormality in the gastro-intestinal tract. Contrasting remarkably with this case was the postoperative course in Experiment 5 in which the lesion in the hypothalamus was non-haemorrhagic and identical with that produced in Experiment 7. Yet the animal went steadily downhill and died within 24 hours of the operation. Autopsy in this case revealed multiple mucosal erosions in the body of the stomach. It would appear from our experiments that extensive gastro-intestinal lesions may alter very rapidly the postoperative course of these animals, quite apart from the nature of the hypothalamic lesion produced.

In only 1 case was haemorrhagic vomitus observed. In Experiment 11, which subsequently showed a destructive lesion in the dorsal part of the tuber cinereum, the animal remained prostrate for 24 hours after the operation. Twenty hours after the lesion had been made the monkey began to vomit a clear mucous fluid mixed with clots of coffee-coloured blood. During the next few hours the animal recovered slightly but continued to vomit blood at intervals, and death occurred within 48 hours of operation. At autopsy multiple haemorrhagic erosions were found in the body of the stomach.

A marked polydipsia following operation was observed in several instances but as the animals were being sacrificed at the end of 3 days no reliable measurements of the daily fluid intake and output were obtained.

Occurrence of Gastric Erosions

Three of the 19 animals died while the lesion was being made. Of the 16 that survived the operation 5 animals showed acute mucosal erosions in the stomach. Two of these survived a 3 day post-operative period, although exhibiting a marked disinclination to eat and a general sluggishness in movements. The remaining 3 of the group showing gastric erosions at autopsy died, 2 within 1 day and 1 in 2 days, each after a stormy postoperative course.

In all of the cases the erosions were confined to the body of the stomach, both the anterior and posterior wall. Sometimes the pyloric region was also involved but there was no evident predilection for this part, or for the lesser curvature of the stomach. The erosions were always multiple and haemorrhagic (Fig. 1) and there were often clots of dark blood intermingled with the gastric contents, definitely establishing the ante mortem nature of such lesions. Some

of the erosions were 5 mm. by 5 mm. and showed a punched-out appearance with slightly raised margins and a haemorrhagic base (Fig. 2), but all were evidently of very recent origin. Microscopical examination revealed that the lesions were all confined to the mucosa and in no case was the muscular layer involved. The histological picture was one of sharply localised destruction of the mucosa with haemorrhagic exudate and no evidence of thrombosis in the sub-mucosal vessels underlying the lesion.

In three of the five experiments in which gastric erosions were present the stomachs of the animals showed considerable dilatation and atony on opening the abdomen. In another animal, in which the lesion had involved the posterior hypothalamic region and yet showed no pathological changes in the gastro-intestinal tract, a similar condition of the stomach was observed at autopsy. This marked dilatation of the stomach might conceivably be attributed to sympathetic hyperactivity. However, we have since seen similar very dilated stomachs in several animals sacrificed after various kinds of cerebral but non-hypothalamic operations, and its significance is therefore not entirely clear.

In the control series of over 50 animals subjected to many types of non-hypothalamic cerebral lesions and sacrificed in this laboratory during the past 6 months, careful postmortem examination of the gastro-intestinal tracts has revealed only 1 case with gastric or duodenal ulceration. This animal was a young capuchin in which the right and left motor and premotor areas had been extirpated 5 months previously. Five weeks before autopsy a transection of the spinal cord at the sixth thoracic level had been performed and 12 days prior to sacrifice the left posterior quadrant of the spinal cord had been divided at the third cervical level. At autopsy the stomach showed three small haemorrhagic erosions, one close to the oesophageal orifice and two in the pyloric end. The contents of the stomach were mucous and mixed with small particles of black blood. The small and large intestine were normal.

This case, in which interruption of sympathetic or parasympathetic pathways from higher centres undoubtedly played a significant rôle in the development of the gastric erosions, stands out as an isolated example from a large series of monkeys that had been subjected to many and varied types of non-hypothalamic cerebral injury and revealed no gastro-intestinal pathology at autopsy. The

consistently negative findings in the long control series of experiments continued from Watts' and Fulton's investigation, lend greater significance to the association of gastro-intestinal lesions with injury to the hypothalamus or interruption of its descending autonomic pathways.

We were impressed at autopsy of primate material with the frequent finding in the large and small bowel, and in one instance in the mucosa of the stomach, of lesions of parasitic origin which often closely simulated chronic peptic ulceration, and it was sometimes necessary to resort to histological methods in order to differentiate the conditions. Tuberculous ulceration of the intestine was also encountered in several instances, but it would seem that true peptic ulceration is a very rare occurrence in normal monkeys.

Site of Lesions in the Hypothalamus

Serial sections showed that in most of the cases the lesions had been made in the tuber cinereum. In 3 cases the posterior hypothalamic region only had been damaged, and in 1 case the lesion had destroyed the supra-optic nucleus on one side. The pre-optic region was never involved.

Of the 5 animals showing well marked gastric erosions at autopsy, all showed lesions confined to the infundibulum or to the grey matter immediately dorsal to it, in which lie the nucleus hypothalamicus periventricularis (anterior and posterior) and nucleus hypothalamicus ventro-medialis* of Papez and Aronson.⁸ The nuclei tuberis lateralis were destroyed in all 5 cases, and in 2 animals there was considerable involvement of the pars tuberalis of the pituitary body, which in the monkey wraps round the base of the infundibular stalk and is in part embedded in the substance of the tuber cinereum. In none of the 5 cases did the lesion encroach upon the nucleus paraventricularis, nor was the nucleus mammillo-infundibularis (hypothalamicus lateralis of Papez and Aronson⁸) involved to any extent. In only 1 of the 5 was the track of the lesion haemorrhagic, and in all 5 cases the base of the third ventricle had been opened.

The precise localisation of the lesion in the 5 cases showing gastric erosions can be summarised briefly as follows.

* The ventral or "principal" nucleus of the tuber cinereum.

EXPERIMENT 4. *Operation January 19, 1934; Slow Postoperative Recovery; Disinclination to Eat; Sacrifice 3 Days Later; Three Small Ulcers in the Body of the Stomach*

Brain Lesion: In sections through the optic chiasma the supra-optic and paraventricular nuclei on each side contained many "shadow" cells, pale and partly disintegrating, and there was a fairly extensive gliosis present. In more caudally placed sections the oral part of the tuber cinereum appeared intact and there was no haemorrhage to be seen. At the point of origin of the infundibular stalk the base of the third ventricle was widely opened from below, the stalk apparently having been separated completely from the base of the brain by the injury. There was no evidence of haemorrhage anywhere. The track of the lesion could not be traced, owing to the destructive nature of the injury, but it appeared to have been uniformly bilateral and the tuber cinereum, including the nucleus tuberis on each side, had been completely destroyed. The posterior hypothalamic region and corpora mammillare were intact.

EXPERIMENT 5. *Operation January 26, 1934; Death on the Following Day; Multiple Gastric Erosions*

Brain Lesion: The track of the lesion could be seen entering the tuber cinereum from the right side, immediately rostral to the base of the infundibular stalk. There was very extensive destruction of the tuberal tissue at this point and the base of the third ventricle was opened. The lesion passed across to the opposite side, but was much more extensive on the right. There was no haemorrhage to be seen in any of the sections. The injury was confined to the nucleus tuberis and the grey matter in the floor of the third ventricle in the immediate vicinity of the tuber cinereum. The supra-optic, paraventricular and mammillo-infundibular nuclei were not involved and the posterior hypothalamic region was intact.

EXPERIMENT 9. *Operation February 5, 1934; Death on the Following Day; Large Dilated Stomach with Multiple Mucosal Erosions*

Brain Lesion: The track of a circumscribed haemorrhagic lesion could be traced, entering the lateral aspect of the tuber cinereum from the left side, just rostral to the commencement of the pituitary stalk. The base of the third ventricle was opened and there was

considerable damage to the right lateral wall. A clot of blood overlapping the optic chiasma extended backwards on each side of the infundibular stalk. There was also a small haemorrhage in the cavity of the third ventricle, around the lacerated right lateral wall. The damage was confined to the tuber cinereum bilaterally and involved the pars tuberalis of the hypophysis. The supra-optic, paraventricular and posterior hypothalamic nuclei were intact.

EXPERIMENT II. *Operation February 8, 1934; Death Within 48 Hours; Dilated Atonic Stomach with Multiple Mucosal Erosions*

Brain Lesion: The lesion was very similar to that in Experiment 9, but had been placed more dorsally. The instrument had entered the hypothalamus on the right side above the tuber cinereum and had pierced the right lateral wall of the third ventricle without much involvement of the opposite side. There was considerable tissue destruction but no demonstrable haemorrhage along the track of the lesion. The grey matter in the floor of the third ventricle, comprising the nuclei hypothalamicus periventricularis et ventro-medialis, had been extensively destroyed but the origin of the pituitary stalk was relatively intact. The nucleus mammillo-infundibularis on the right side was damaged but the rest of the hypothalamic nuclei were uninvolved.

EXPERIMENT 15. *Operation February 26, 1934; Good Postoperative Recovery; Polydipsia and Disinclination to Eat; Sacrifice 3 Days Later; Multiple Haemorrhagic Erosions in the Body of the Stomach*

Brain Lesion: The lesion was entirely unilateral and confined to the oral parts of the tuberal region. The supra-optic nucleus and the nuclei hypothalamicus periventricularis et ventro-medialis on the left side had been damaged; there was no involvement of the mammillo-infundibular nucleus or the posterior hypothalamic region. No evidence of haemorrhage could be seen in any of the sections.

DISCUSSION

Positive evidence is brought forward here to show that in monkeys histologically verified lesions, *confined to the tuberal nuclei*, leaving all other hypothalamic nuclei intact, may lead to haematemesis and

multiple mucosal erosions in the body of the stomach. Several other cases with brain lesions, identical in localisation and extent, were unassociated with any recognisable gastro-intestinal pathology. It would appear that an irritative process caused by haemorrhage at the site of the hypothalamic injury is not a deciding factor in the production of gastric erosions, since such haemorrhage in the hypothalamus occurred in only 1 out of the 5 cases showing erosions, and since several cases with extensive haemorrhagic lesions revealed no evidences of pathology in the gastro-intestinal tract. In three experiments in this series with lesions confined to the posterior hypothalamic region, at autopsy no erosions were found in the stomach, but the cases are too few to warrant any statement regarding the relation of the posterior hypothalamic nuclei to gastric ulcer.

In the series of Watts and Fulton⁶ the injury in most cases involved the tuberal, supra-optic and paraventricular nuclei and, as in our series, no case of gastric erosions was encountered in association with posterior hypothalamic injury. In the experiments of Burdenko and Mogilnitzki⁴ the injury was made immediately behind the infundibular stalk, and in those of Keller, Hare and d'Amour⁵ at the level of the optic chiasma. The association of tuberal lesions with gastro-intestinal ulceration seems therefore established.

Two interpretations have been advanced in the consideration of the neurogenic factor in the genesis of experimental gastric ulcer, one based on a conception of sympathetic hyperactivity, the other explaining the phenomena on grounds of parasymphathetic hyperactivity.

(1) *Sympathetic Hyperactivity*

The Peripheral Mechanism: According to this view the sequence of events which leads to the production of gastric ulceration is believed to be overactivity of the sympathetic vasoconstrictors, spasm of the terminal vessels in the submucosa of the gastro-intestinal tract, with the production of multiple areas of ischaemia in the mucosa and digestion of the necrosed tissue by the acid gastric secretions, leaving small punched-out erosions.

The Central Mechanism: In order to explain on the basis of this theory the association of erosions with hypothalamic injury, it is

necessary to consider either a "release" or an "irritation" of the sympathetic centres in the hypothalamus. The development of erosions within 24 hours of the brain injury can be interpreted equally well on the basis of an irritative or of a release mechanism, either of which can be assumed to exist immediately following the injury. It is, however, rather difficult to understand how the small lesions in the present series, which were confined to the tuberal nuclei, could have interrupted any great number of inhibitory fibres from higher centres.

(2) *Parasympathetic Overactivity*

The Peripheral Mechanism: Cushing¹ has summarised the possible mechanism by which hyperactivity of the parasympathetic apparatus may act in the production of gastric ulceration as follows: "Direct stimulation of the tuber or of its descending fibre tracts, or what theoretically amounts to the same thing, a functional release of the vagus from paralysis of the antagonistic sympathetic fibres, leads to hypersecretion, hyperchlorhydria, hypermotility and hypertonicity especially marked in the pyloric segment. By the spasmodic contractions of the musculature, possibly supplemented by accompanying local spasms of the terminal blood vessels, small areas of ischaemia or haemorrhagic infarction are produced, leaving the overlying mucosa exposed to the digestive effects of its own hyperacid juices."

The Central Mechanism: Hypothalamic lesions may lead to overactivity of the parasympathetic system either by direct destruction of the sympathetic centres, thus releasing the vagus from the antagonistic sympathetic activity, or by irritation of parasympathetic pathways in this part of the brain. Beattie and Sheehan⁹ showed that faradic stimulation of the anterior part of the hypothalamus in the fasting cat resulted in parasympathetic phenomena, *i.e.* in a rise of intragastric pressure which was followed by an increase of peristaltic movements of the stomach.

The lesions associated with gastric erosions in the present series of experiments were confined to the tuberal nuclei and it is difficult to see how they could have caused any marked destruction of the sympathetic centres which extend backwards into the posterior hypothalamic region. If the anterior hypothalamic region can be assumed tentatively as the location of parasympathetic discharge (Cushing,¹

Beattie¹⁰), then the evidence here is suggestive of an irritation of such parasympathetic pathways as the underlying mechanism, since all the lesions were small and superficial in extent. Functional localisation in the hypothalamus is however not sufficiently clarified to warrant any final conclusion from the present study as to the exact mechanism involved. A study of the experimental production of gastric ulcer following *peripheral* nerve lesions shows that splanchnotomy is by far a more frequent cause than vagotomy (Cushing¹) although vagal section has led to acute ulceration in the stomach and duodenum in some cases (Ferguson¹¹).

We have attempted to throw more light on the question by a pharmacological approach to the problem. For this purpose monkeys (*Macaca mulatta*) have been subjected to repeated subcutaneous injections of (a) acetyl- β -methylcholine chloride in amounts of 25 to 50 mg. per kg. body weight, or (b) adrenalin 0.5 cc. of a 1:1000 solution per kg., or (c) adrenalin 1.5 cc. of a 1:1000 solution per kg., with atropine 20 mg. per kg., or (d) pituitrin (surgical) 1 to 3 cc. (The injections of pituitrin were given in view of Dodds, Noble and Smith's¹² recent findings in rabbits.) The doses were given in each experiment every 2 to 3 hours for periods of 3 to 7 days. In no case were there observed at autopsy any erosions of the gastric mucosa comparable to the results obtained from hypothalamic injury. Although these negative findings cannot be accepted as conclusive evidence, and the cases are too few in number, nevertheless they indicate that states of profound overactivity of the sympathetic or of the parasympathetic system may exist for comparatively prolonged periods without the appearance of gastric erosions.

In considering the neurogenic influences in the production of gastro-intestinal lesions it must not be lost sight of that a vasomotor disturbance is only one of many factors that undoubtedly plays an important rôle. The motility of the gastro-intestinal tract and the amount and nature of the digestive juices are under the direct influence of the central nervous system. The influence of the vagus nerve on gastro-intestinal motility is usually looked upon as due to an augmentation of peristalsis and an inhibition of sphincteric action, while the sympathetic innervation to the gut is considered to act in an antagonistic manner. McSwiney¹³ considers, however, that it is possible to postulate the presence of motor and inhibitory fibres to the stomach in both the vagus and sympathetic nerves, and

that the immediate effects of vagotomy and splanchnotomy are similar, namely retardation of function. It is highly probable therefore that there is no real fundamental opposition in the two views that have been advanced to account for the occurrence of gastro-intestinal lesions of nervous origin, and that the explanation may lie in a complex imbalance between the sympathetic and parasympathetic systems.

SUMMARY AND CONCLUSIONS

1. In 16 monkeys, following hypothalamic injury, 5 animals showed multiple haemorrhagic erosions in the mucosa of the body of the stomach.

2. The animals showed striking individual variations in the general postoperative condition. Those with gastro-intestinal lesions showed a disinclination to eat, their condition became progressively worse, and in three experiments death supervened in 24 to 48 hours.

3. In all of the cases the erosions were confined to the stomach, none occurring in the duodenum. The erosions were multiple and haemorrhagic, entirely confined to the mucosa, and some showed a punched-out appearance.

4. In three of the five experiments in which gastric erosions were present the stomachs showed considerable dilatation and atony, suggestive but not conclusive evidence of sympathetic activity.

5. Histological examination of the hypothalamic injuries revealed that in all the animals showing gastric erosions at autopsy the lesions were small and confined to the tuberal nuclei. In only 1 of the 5 was the track of the injury haemorrhagic. Positive evidence is therefore advanced to show that histologically verified lesions, confined to the tuberal nuclei and leaving all other hypothalamic nuclei intact, may lead to haematemesis and multiple mucosal erosions in the body of the stomach.

6. In a control series of over 50 monkeys subjected to many and varied types of non-hypothalamic cerebral lesions careful autopsy examination of the gastro-intestinal tracts revealed only 1 case with gastric or duodenal ulceration, and this animal had been subjected to a bilateral motor and premotor extirpation 5 months prior to sacrifice and to transections of the spinal cord at the sixth thoracic and third cervical levels 5 weeks and 12 days (respectively) before autopsy. The consistently negative findings in the control experi-

ments would appear to lend greater significance to the association of gastro-intestinal lesions with injury to the hypothalamus or interruption of descending autonomic pathways.

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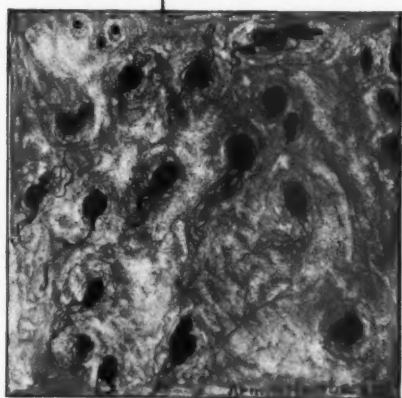
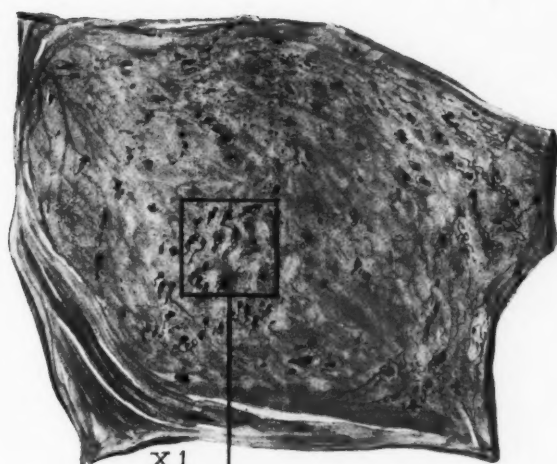
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DESCRIPTION OF PLATES

PLATE 113

FIG. 1. Drawing to show the naked eye appearance of the mucosal surface of the stomach when first opened. The erosions are multiple and haemorrhagic. (Experiment 9. Capuchin. Death within 24 hours after a hypothalamic lesion in the tuber cinereum.)



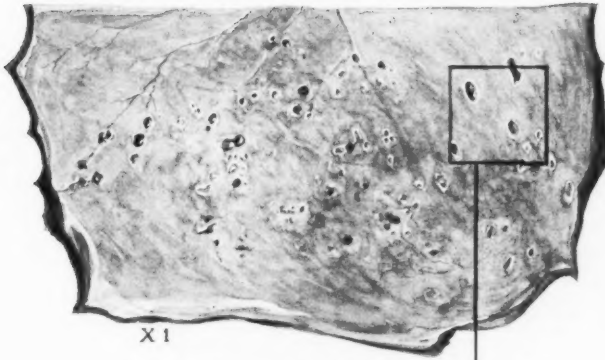


X 4

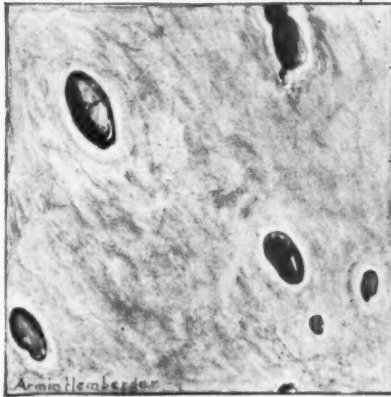
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PLATE II4

FIG. 2. Drawing to show the punched-out appearance of the gastric erosions, the bases of which are haemorrhagic. All were of recent origin and occurred chiefly on the dorsal wall of the stomach, extending from the oesophageal opening to about 2 cm. from the gastroduodenal junction. (Experiment 5. Capuchin. Death within 24 hours after a hypothalamic lesion confined to the tuberal nuclei.)



X 1



X4

2



FUNCTIONAL COR TRILOCULARE BIATRIA *

REPORT OF A CASE WITH A MALPOSITION OF THE SEPTUM IN THE VENTRICLES

DANIEL KORNBLUM, M.D.

(From the Department of Pathology of the Brooklyn Jewish Hospital, Brooklyn, N. Y.)

Cases of malposition of a defective septum in the ventricles, so that both atriums empty into a common chamber, appear to form a definite entity. Twelve cases have been reported in the literature by Holmes,¹ Peacock,² Favorite,³ von Rokitansky⁴ (2 cases), Thérémín,⁵ Chiari,⁶ Mann,⁷ Marchand,⁸ Mills⁹ and Spitzer¹⁰ (2 cases). These hearts have been designated as cases of functional cor triloculare biatria. In all these cases the septum was placed to the right, separating a small right ventricle from a large left ventricle in which both auriculoventricular orifices were present. In the first case reported (Holmes¹) the pulmonary artery arose from the small right chamber. In the remaining cases the aorta arose from the latter in transposed relation to the pulmonary artery. The writer has had the opportunity to study a heart which is unique in that the septum was displaced to the left instead of to the right. The aorta arose from the small left chamber and was in a transposed relation to the pulmonary artery, which sprang from the large right ventricle. The right chamber contained both venous orifices. With certain associated anomalies this heart produced a complex picture, and an attempt will be made in this paper to trace its development in the light of recent researches on the embryology of the heart.

REPORT OF CASE

Clinical History: Baby girl, K. M., aged 5 months, was admitted to the Pediatric Service of the Brooklyn Jewish Hospital on Nov. 1, 1931, with a history of malnutrition since birth, cough and coryza of 1 weeks duration, and cyanosis of the lips for 2 days.

The mother of the child was 30 years of age and in good health. She had one other child, aged 7 years, living and well. Following the birth of this baby, she had three miscarriages, the first two at 3 months and the third at 4 months. She then gave birth to a full term infant who had a large head and died in convulsions 5 hours postpartum.

* Received for publication March 6, 1935.

The patient was born spontaneously at full term. There was no history of any birth injury but she was markedly cyanotic at birth and was resuscitated with difficulty. Her birth weight was 7 pounds 4 oz. She was ill since birth and failed to gain weight, taking all her feedings poorly, each of which was vomited, the vomiting sometimes being projectile in type. When the baby was 2 months of age a physician said she had an enlarged heart, which was confirmed by fluoroscopy. She always had very cold extremities. Two weeks before admission she developed a cough and coryza which persisted, but cyanosis, absent since birth, did not reappear until 2 days before admission.

On admission, examination revealed an acutely ill, marasmic infant, slightly cyanotic. A mucopurulent discharge from the nose was present and the pharynx was very congested. There was a diminished percussion note over the left chest. Bronchial breathing and numerous crepitant râles were present in several parts of the lung. The right border of the heart was percussed at its farthest point, 3 cm. from the midline, and the apex 1.5 cm. to the left of the left midclavicular line in the fifth interspace. The apex beat was diffuse and heaving, and a palpable systolic thrill was present over the entire precordium, but most marked at the apex. The heart sounds were of good quality, rapid and regular. A loud, moderately high pitched, rough systolic murmur was heard over the entire precordium, being most marked at the apex. The spleen and liver were not palpable. The extremities were very cold. No deformities were present. A clinical diagnosis of congenital heart disease, acute nasopharyngitis, acute bronchopneumonia, and marasmus was made. A blood transfusion was given, but the child grew progressively worse and died Nov. 13, 1931, after 12 days in the hospital. The temperature throughout remained normal and subnormal, except for an ante mortem rise. Cyanosis was never marked.

The blood count was 3,880,000 erythrocytes, 20,500 leukocytes, 76 per cent polymorphonuclear neutrophils and 22 per cent lymphocytes. The hemoglobin was 58 per cent (Dare). The urine showed 1 plus albumin. Tuberculin, Schick, Wassermann and Kahn tests and nose and throat cultures were negative.

X-rays of the chest revealed a bronchopneumonia. The cardiac shadow was reported as broadened slightly to the right and considerably to the left, the general condition suggesting the presence of congenital heart disease. There was no evidence of luetic changes in the X-ray plates of the extremities.

Electrocardiographic studies, unfortunately, were not made.

AUTOPSY REPORT

The body is that of a well developed, emaciated female infant of 6 months, 60 cm. in length, weighing 7 lbs. and 9 oz. The skin is slightly cyanotic. The thymus weighs 2 gm. and appears normal on section. Both lungs show numerous, well demarcated patches of reddish brown color which microscopically show exudation of fluid, fibrin, red blood cells and leukocytes into the alveoli. The remaining organs show varying degrees of congestion and cloudy swelling of the parenchyma.

Heart: The heart weighs 4 gm. Notes on the position of the heart

in the body are lacking. Viewed anteriorly, the organ appears as a right angle triangle with the right angle in the upper left portion where the aorta emerges. The left border of the ventricle, 6 cm. long, descends almost vertically while the right border, 7.5 cm. long, curves downward from right to left forming the hypotenuse. The heart appears rather pale red in color, is firm in consistence, and there is a diminished amount of fat in the auriculoventricular sulcus. The visceral and parietal pericardiums are smooth and glistening, and no defects or adhesions are present. There is a normal quantity of clear fluid in the pericardial sac.

The atriums are present in their normal position, and their appendages curve normally on either side of the great vessels. The largest circumference of the right atrium is 4 cm. The entrances of the superior vena cava, inferior vena cava and the coronary sinus are in their normal relations, and no anomalies of the venous valves are present. There is a slight valvular patency of the foramen ovale. The left atrium is 4.5 cm. in its largest circumference and shows no pathological changes. It receives four pulmonary veins in their normal relations.

On opening the ventricles posteriorly, it can be seen that the septum is displaced to the left, dividing approximately the right four-fifths of the heart from the left one-fifth, producing a small chamber on the left and anteriorly (which gives off the aorta) and a large chamber on the right and posteriorly. In the latter, both auriculoventricular orifices and the orifice of the pulmonary artery are present (Fig. 1).

The ventricle of the left side is reduced in size to a small cone 1.5 cm. long, the base of which is occupied entirely by the root of the aorta which is 2 cm. in circumference. The trabeculae on the anterior wall are prominent. Those posteriorly are flattened and the septal wall is smooth.

The ventricle on the right side is about four times the size of that on the left and is also conical in shape. The myocardium measures 1 cm. at its thickest portion near the base posteriorly and is thinned to 4 mm. near the apex. There can be distinguished two papillary muscles in close approximation on the anterior wall, one on the lateral, and two on the posterior wall. On the septal wall there is a low band of muscle 0.5 cm. wide and 2 cm. long, continuous with the aortopulmonary septum, and coursing downwards, anteriorly,

toward the right, to end in the anterior wall. The remaining portions of the inner aspect of the ventricular walls are traversed by fine interlacing strands of muscle and fibrous tissue, except for the septal surface which is smooth.

The auriculoventricular orifice on the right side is 3.5 cm. in circumference and is occupied by a large medial cusp, the base of which is attached to the free edge of the interauricular septum, and a long, narrow, lateral cusp in which individual cusps cannot be identified. The medial cusp is attached anteriorly to the medial of the two anterior papillary muscles, and posteriorly to the muscle on the lateral wall. The lateral cusp is attached to both the two posterior and the lateral muscles. On the left side there is also a medial and a lateral cusp. The former is attached to the free edge of the interatrial septum on a base in common with that of the medial cusp of the right side. Posteriorly the former cusp is connected to the right posterior papillary muscle, while anteriorly it is joined to the superior wall of the septal defect (the aortopulmonary septum) by short chordae. The lateral cusp is similarly attached anteriorly, while posteriorly it is connected to the large, left, posterior muscle.

The septum (Fig. 1) is roughly triangular in shape, 2.5 cm. wide at the base, 5 cm. long, 0.5 cm. thick, and is curved slightly from left to right, so that its concavity looks to the right and posteriorly. Its apex is on the left border of the heart about 1.5 cm. from the apex of the heart. The base is attached to the ventricular walls by an anterior and a posterior root. The former is attached to the right auriculoventricular orifice at the anterior end of the common base of the medial cusps of both auriculoventricular openings. The posterior root is attached to the posterior ventricular wall, caudal and to the left of the anterior attachment. Between these two roots the base of the septum forms a free falciform edge. The aortopulmonary septum from above meets this edge in the following way: anteriorly the former is in a plane slightly more anteroposterior than that of the interventricular septum, and thus its attachment to the anterior ventricular wall is to the left of the anterior root of the septal base. A triangular depression (Fig. 1) is thus formed on the anterior ventricular wall between the aortopulmonary septum on the left and the anterior root of the interventricular septum on the right. The orifice of the pulmonary artery is bounded anteriorly by the base of this triangular depression, and posteriorly by the common base of

the medial auriculoventricular cusps. Posteriorly the aortopulmonary septum is the plane of the interventricular septum, but fails to join with it, resulting in a defect 1.2 cm. in diameter which connects both ventricles. This defect is roughly diamond-shaped and completely surrounded by muscle, above by the aortopulmonary and below by the interventricular septum.

The pulmonary orifice (Fig. 1) is to the right and posterior to the aortic orifice. The great vessels maintain this relation as they course distally. No torsion is present. Thus, distally they are in their normal relation, while proximally their normal positions are reversed.

The semilunar cusps are normal in size, shape and number. The non-coronary cusp is placed anteriorly.

The coronary arteries and veins are not injected, and only the larger vessels can be distinguished. The right coronary artery emerges from the right aortic sinus and gives off immediately in the auriculoventricular sulcus a large branch, which descends toward the left on the anterior surface of the heart to the apex. Near its origin this branch gives rise to another branch which descends toward the right, dividing into several tributaries. The artery continues in auriculoventricular sulcus around the right border of the heart, giving off in its course several short branches which descend toward the apex.

The left coronary artery arises from the left aortic sinus and courses toward the left in the auriculoventricular sulcus for 1.5 cm. where, at the junction of the left atrium and its appendage, it gives off a large branch which descends on the posterior surface toward the left border and the apex. It divides into several branches near the apex, which course around the left border to mingle with the branches of the anterior descending artery. The remaining small portion of the artery courses in the auriculoventricular sulcus toward the right coronary, giving off several short descending branches. No abnormality was noted in walls of the vessels.

The coronary sinus courses posteriorly in the auriculoventricular sulcus, toward the left border.

Microscopic examination of the heart muscle revealed no lesions. No attempt was made to trace the course of the conduction system.

DISCUSSION

Let us first direct attention to the corrected transposition of the great vessels that exists in this heart. By corrected transposition of the main trunks is meant that condition in which the two vessels exist in reverse relation to each other (the aorta being placed anteriorly instead of posteriorly in relation to the pulmonary artery) yet spring from the ventricles to which they normally belong. Examples of this condition are of rare occurrence, there being only 21 reported in the literature. Robertson,¹¹ Sato¹² and Spitzer¹⁰ have accounted for their occurrence by supposing that a situs inversus of the ventricles occurs with a transposition of the great vessels (the atriums remaining in their normal position), so that the right or tricuspid ventricle is formed on the left side but still gives rise to the aorta; and similarly, the left or bicuspid ventricle forms on the right side and gives rise to the pulmonary artery. Von Rokitsky⁴ labelled these cases as corrected transposition of the great vessels, since he did not draw the distinction between bicuspid and tricuspid ventricles, calling a ventricle on the right side the right ventricle, even though it had a bicuspid valve and no conus. He believed that the correction was due to a sympathetic adjustment of the interventricular septum with the bulbar septum, so that the great vessels opened into their proper ventricles. However, the view that corrected transposition is produced by an independent situs inversus of the ventricle loop seems well established, especially since in the reported cases there is characteristically a reversal of the auriculoventricular cusps and an absence of the conus arteriosus in the right ventricle. In a heart described recently by Walmsley¹³ there is additional evidence of an independent situs inversus of the ventricles besides a reversal of the auriculoventricular cusps which, alone, cannot be taken as good evidence. Walmsley lists, in describing his heart, a reversal of the internal conformation of the ventricles, a reversal in the forms of the auriculoventricular bundle, a reversal of the coronary artery fields, and a left-sided position of the interventricular septum, so that the pars membranacea is placed between the left atrium and right ventricle, instead of between the right atrium and left ventricle. Thus, because of the presence of a corrected transposition of the great vessels, it is believed that a ventricular situs inversus exists in this heart. Additional evidence

of this condition will be seen in studying the other existing malformations.

Let us turn now to a consideration of the development of the septum on the right side, as was present in the reported cases of functional cor triloculare mentioned above. Cases have been described in which there was also a septum separating the conus from the sinus of the right ventricle. This septum is explained by Keith¹⁴ as a persistence of the lower bulbar orifice. It exists ordinarily in reptiles. It usually shows much fibrous thickening in the human heart, and so it has been thought to be due here to inflammatory contraction of the conus. However, the septum may be muscular, as in a case reported by Bohm,¹⁵ in which the sinus was separated from the conus by a muscular ridge. This septum may also exist with an interventricular septum, as in the 2 cases reported by Mackenzie¹⁶ of hearts with "three ventricles," and in a case reported by Dudzus¹⁷ of a heart with "four ventricles." In the hearts with functional cor triloculare reported by Young¹⁸ and Peacock,² in addition to the right-sided septum, a low muscular ridge was present in the normal position of the interventricular septum. Thus, these authors considered that the right-sided septum represented the persistent lower orifice of the bulbus cordis and had grown entire, separating the two portions of the right ventricle, while the development of the interventricular septum had been arrested. Spitzer¹⁰ believed that the right-sided septum in his cases, called by him "mixed transposition" of the great vessels, was a septum spurium formed by the fusion of the hypertrophied crista supraventricularis and the anterior tricuspid ridge, while the true interventricular septum was rudimentary or entirely absent. His theory fails to account for the condition in the heart reported by Holmes, mentioned above, in which the malposition of the septum was present without a transposition of the vessels.*

Abbott¹⁹ made a study of the heart reported by Holmes and a similar heart in the museum of the Harvard Medical School, and came to the conclusion that, in these specimens at least, the strong muscular wall with a large defect at its upper border through which

* Furthermore, as Walmsley points out, in corrected transposition of the vessels Spitzer considers the anterior part of the interventricular septum to be formed from the crista supraventricularis, although the latter should be formed posteriorly on account of the presence of the situs inversus of the ventricles, which Spitzer believes exists in these cases.

the small cavity communicated with the large common ventricle, was simply the malposed interventricular septum. Similarly, in the heart reported here, that the septum was the true interventricular septum is strongly evidenced by the fact that it was a strong muscular wall with a free falciform edge at its base which, anteriorly, was in relation to the anterior endocardial cushion, and posteriorly to the right bulbar ridge, the conditions closely resembling those present in the embryo (Fig. 2). It is evident that the malposition was either due to a growth of the septum primarily in its position to the right of the right auriculoventricular orifice, or else was brought there in the development of the heart from its normal or abnormal position.

Abbott considered that a septal defect, produced by failure of union of the aortic with the interventricular septum, would, as she stated in 1901,²⁰ bring the blood of both atriums to the left ventricle which, thus having an increased amount of work, would enlarge greatly and bring the defective septum, in the further development of the heart, to the right. It is not clear from this description why the septum should be displaced to the right side of the tricuspid orifice from the left side where it was attached. Interventricular septal defects are relatively common, yet they are rarely associated with displacement of septum to the right of the tricuspid orifice, and never with sufficient enlargement of the left ventricle to bring it there. One cannot conceive how, in view of the dynamics of the circulation, the interventricular septum can be brought across a venous orifice in the development of the heart.

In view of these considerations it is necessary to assume that the alternative occurs, namely that the septum develops primarily to the right of the right auriculoventricular orifice. Normally in the early stages of the development of the heart the posterior border of the septum becomes closely attached to the left side of the right auriculoventricular opening (Fig. 2), but if for some reason it had grown only slightly farther to the right it would have been brought into relation with the right bulbar ridge on the right side of the auriculoventricular opening. Then if development continued the usual way, the bulbar ridges fusing with each other and with the interventricular septum, the right auriculoventricular ostium would remain in the left ventricle along with the mitral orifice, and there would be present on the right side a small chamber without a venous orifice. In most of the reported cases of this condition detailed de-

scriptions are unfortunately lacking of the region of the attachments of the interventricular and aortopulmonary septums, and little evidence can be adduced from them in support of the above hypothesis.

The anatomical configuration of the heart reported here indicates that the position of the interventricular septum has been the result of a process such as that described above, but in the presence of a situs inversus of the ventricles. The anterior attachment * of the base of the interventricular septum remains in its normal position at the anterior end of the common base (Fig. 1) of the median cusps which represents in this heart the fused primitive auriculoventricular cushions. However, the posterior attachment, instead of being to the right of the left auriculoventricular orifice in relation to the posterior end of the common base of the median cusps, *i.e.* the primitive posterior endocardial cushion, becomes placed to the left of the left venous ostium joining with the posterior bulbar ridge.† The falciform edge which forms the inferior border of the primitive interventricular foramen can be traced in this heart between these two attachments (Fig. 1), so that the foramen resembles its early form except that, due to the left side displacement of the posterior attachment, it is in a plane placed obliquely from right anterior to left posterior, instead of the almost sagittal plane in which it is normally placed. The aortopulmonary septum, due to its malrotation, becomes placed in a plane more anteroposteriorly than the septum, so that the anterior ridge becomes attached to the left of the anterior root of the interventricular septum, instead of coming in direct relation to it (Fig. 1). Thus, there exists a condition which represents an arrest in development from the embryonic heart, *i.e.* the blood passes over the base of the interventricular septum and in front of the fused endocardial cushions to reach the pulmonary channel, which normally would be the aortic channel. The remaining portions of the aortopulmonary and interventricular septa fail to fuse, so that the two bulbar ridges superiorly, and the interventricular septum inferiorly, form the roughly diamond-shaped opening between the ventricles (Fig. 1), as described in the heart reported by Holmes. In this way these anatomical peculiarities which exist

* This would correspond to the posterior portion of the septum with the ventricles in their normal position.

† This would be more clearly conceived if one looks at Fig. 2 through a mirror or from the reverse side of the page.

in the heart reported in this paper are explained on a basis of a primary attachment of the posterior portion of the interventricular septum to the left of the left auriculoventricular orifice (instead of to the right of the right orifice if it had taken place in the normal position of the ventricular loop).

The median tendon or papillary muscle which, according to Mall²¹ is the most constant attachment of the tricuspid valve, is present in this heart as the connection between the medial and lateral cusps of the left valve with the aortopulmonary septum. This valve thus corresponds to the tricuspid valve of normal hearts, even though only two cusps can be distinguished. In independent situs inversus of the ventricular loop the auriculoventricular orifices remain in their normal position along with the atria, but since the connection of the median tendon is brought between the left valve and aortic septum, this valve instead of the right one is transformed into a tricuspid valve. The determination of the papillary muscles in this heart is difficult in view of the changes in their position and conformation brought about by the formation of a common ventricle. The two papillary muscles on the posterior wall and muscle on the lateral wall possibly represent, as is seen from their attachments to the right auriculoventricular valve, the anterior and posterior papillary muscles respectively of the normal heart, which are reversed due to the situs inversus. The anterior muscles similarly possibly represent the divided large papillary muscle that exists in the normal heart in the right ventricle. The presence of the non-coronary aortic cusp anteriorly is due to the failure of torsion of the great vessels.

Lewis and Abbott²² attempted to explain the difference that exists between the heart reported by Holmes, without transposition of vessels, and the remaining hearts of this type of cor triloculare which had a complete transposition of great vessels by assuming that the latter hearts were developed upon a reversed ventricular loop producing the transposition of vessels, while the heart reported by Holmes was developed in the normal position. But it is not clear from their models how both variations would produce a septum on the right, as exists in all those hearts. A reversal of the ventricular loop would bring the septum and great vessels to the left, as described in the reported specimen. No evidence is described of a situs inversus of the ventricles in these hearts. It appears, then, that the

process they believed took place in the hearts with complete transposition of the vessels with displacement of the septum to the right, actually took place in this heart, producing a septum on the left and a corrected transposition of the great vessels. In the former hearts, and also in that reported by Holmes, the right-sided septum occurred with the ventricles in their normal position. With the exception of the heart reported by Holmes, transposition of vessels has always occurred with this type of functional cor triloculare. The transposed relationship is frequently present in true three-chambered hearts.

Thus, the major abnormalities that exist in this heart are accounted for, and the steps in their pathogenesis are explained on the basis of a transposition of vessels occurring with a situs inversus of the ventricles and a primary malposition of the interventricular septum.

The clinical manifestations of functional cor triloculare are similar to those of true three-chambered hearts. Both varieties are compatible with fairly long life. The maximum age of the 11 individuals in the cases mentioned above was 23 years, the minimum, 9 weeks. Cyanosis, although at times severe, was usually present only to a slight degree, indicating a relatively small amount of free admixture of venous and arterial blood in these hearts. Clubbing of the fingers was absent or slight. Murmurs were inconstant, and the physical signs were not characteristic. Suffocative attacks were a frequent occurrence. Cardiac decompensation was the usual terminal event.

SUMMARY

A case is presented of functional cor triloculare biatria in which the interventricular septum was displaced to the left, dividing a large right chamber containing both auriculoventricular orifices and the pulmonary artery from a small left chamber which gave rise to the aorta.

The clinical manifestations of this anomolous heart are presented and its pathogenesis is discussed.

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DESCRIPTION OF PLATES

PLATE 115

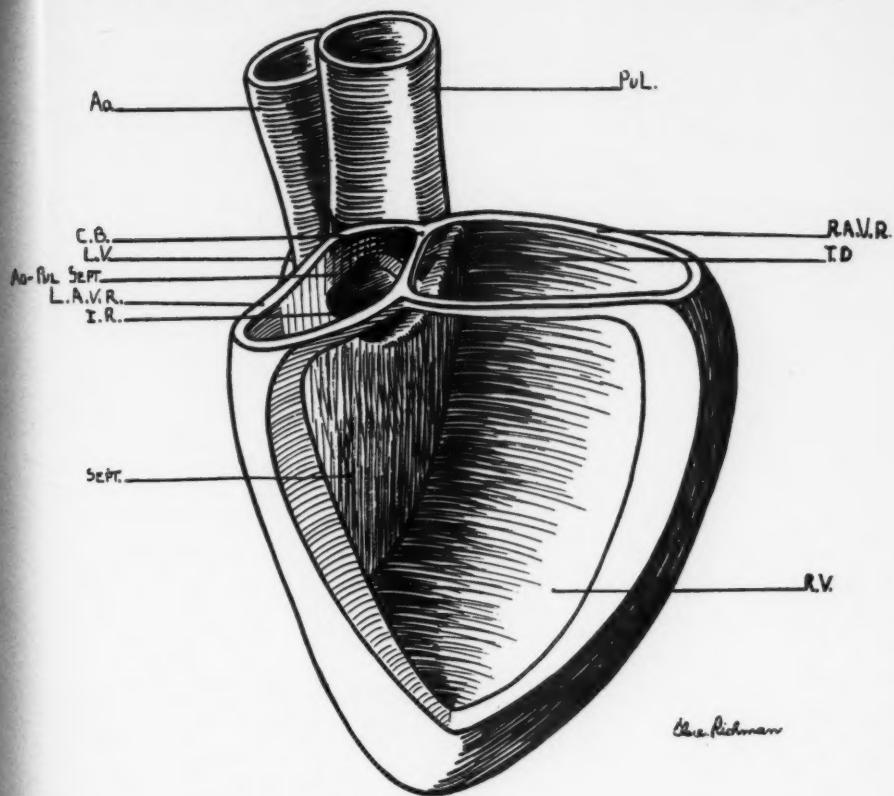
FIG. 1. Diagrammatic drawing of heart viewed posteriorly, the auricles having been removed, leaving the auriculoventricular rings without the valves. A window is cut in the posterior wall of the right ventricle.

Ao. = aorta; Ao-Pul. Sept. = aortopulmonary septum; C. B. = common base of median cusps of both auriculoventricular orifices and free edges of interauricular septum; I. R. = interventricular foramen; L. A. V. R. = left auriculoventricular ring; L. V. = left ventricle; Pul. = pulmonary artery; R. A. V. R. = right auriculoventricular ring; R. V. = right ventricle; Sept. = septum in the ventricles; T. D. = triangular depression.

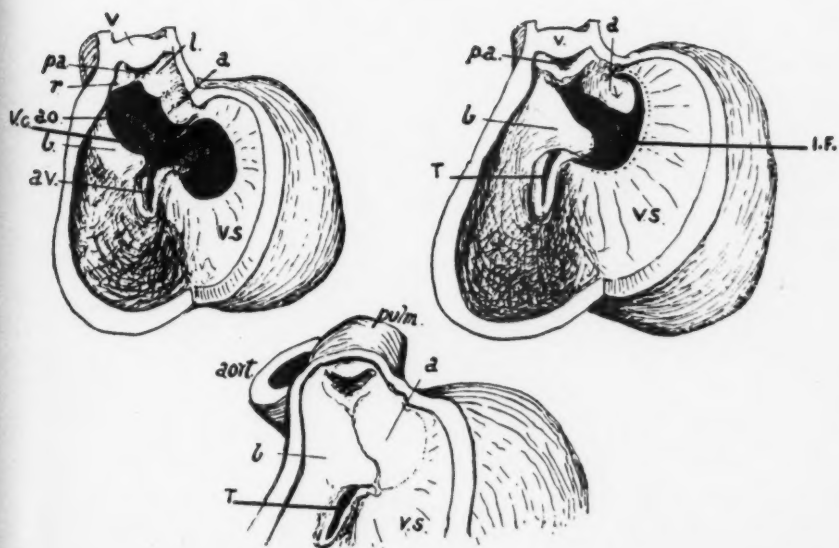
FIG. 2. Showing method of division of bulbar region and formation of aortic vestibule.

a = left bulbar ridge; b = right bulbar ridge; a v. = auriculoventricular orifice; T = right tricuspid orifice; V. S. = ventricular septum; V. C. = ventral endocardial cushion; I. F. = interventricular foramen (from Buchanan²⁸); p a = pulmonary artery; r = right bulbar cushion; l = left bulbar cushion; a o = aortic opening.





I



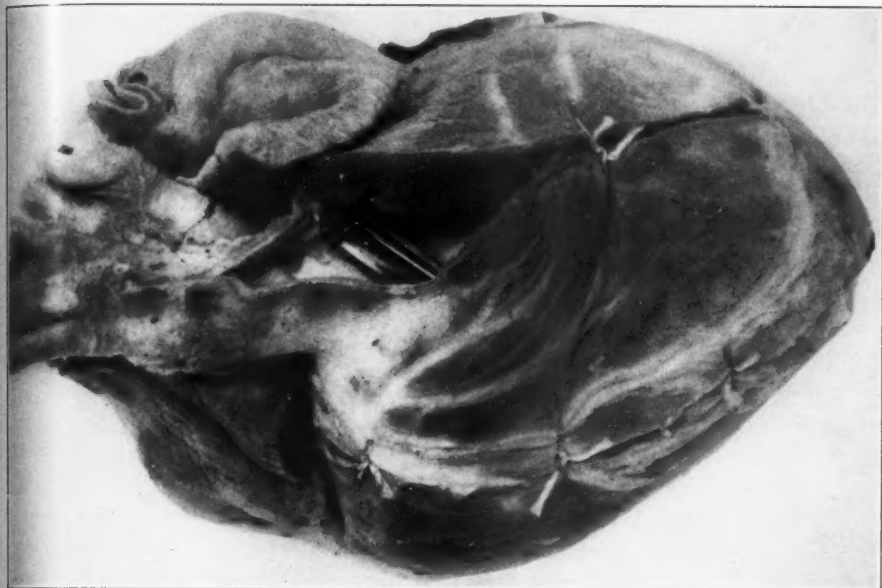
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PLATE 116

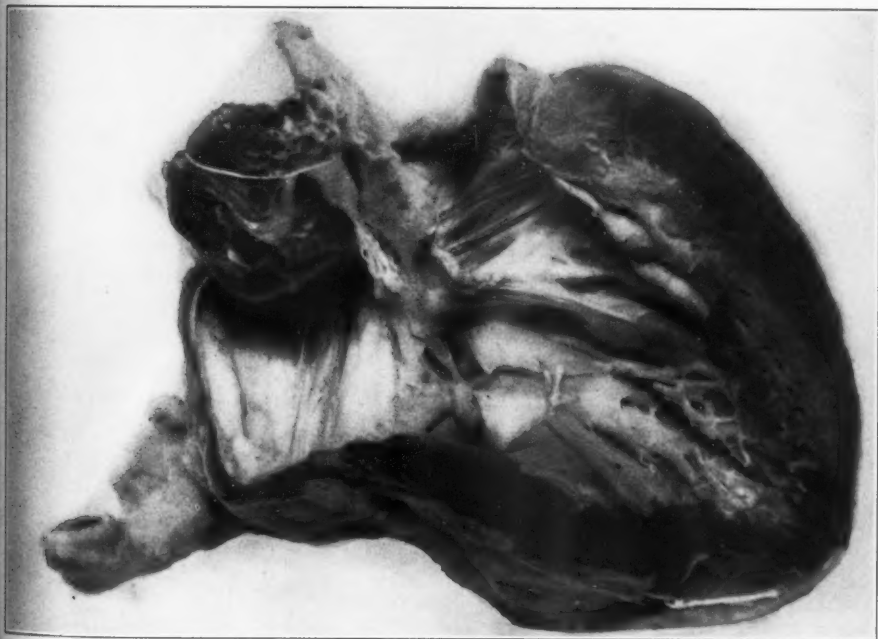
FIG. 3 A. Posterior view of heart with posterior wall elevated, exposing the interior of the right ventricle and both atria.

FIG. 3 B. Left anterolateral view of heart showing interior of left ventricle and aorta.





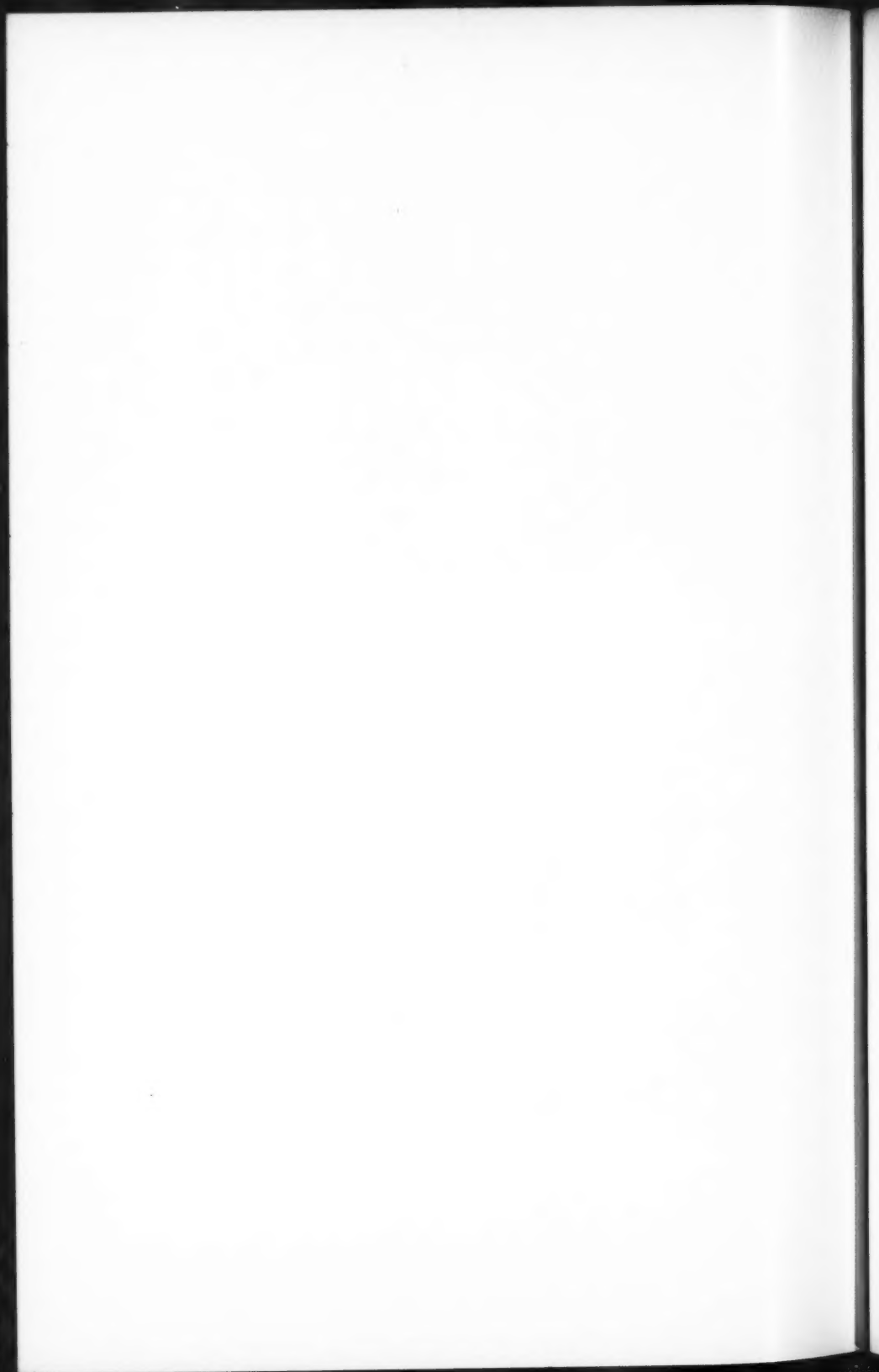
3B



3A

Kornblum

Cor Triloculare Biatría



AN IMPROVED TECHNIQUE FOR SILVER IMPREGNATION OF RETICULUM FIBERS *

HELENOR CAMPBELL WILDER

(From the Army Medical Museum, Washington, D. C.)

The argentation of reticulum fibers as an aid in the classification of tumors has been in use in this laboratory since 1924. Foot's^{1,2} technique has been the routine method employed; Laidlaw's³ method has been used occasionally. It has been previously reported (Wilder⁴) that glia cells in paraffin sections of formalin-fixed tissues may be impregnated, rapidly and without the use of heat, by sensitization with uranium nitrate prior to the exposure to ammoniacal silver (Foot's silver diammino hydroxid). The method presented in this paper is based on Foot's reticulum stain and the sensitization method for glia. It is very rapid and stains the finest reticulum fibers with great precision, collagen appearing rose-colored as in Foot's stain. It is applicable to formalin or Zenker-fixed tissues and to paraffin, celloidin and frozen sections. The problem of preventing sections from washing off the slides in ammoniacal silver has been solved in some measure by reduced exposure to this solution and the elimination of heat. However, the first loosening of the section occurs in the potassium permanganate solution and, even with this new method, occasional sections affixed with glycerine-albumin do not adhere throughout the subsequent procedures. In the staining of these difficult sections it has been found that substitution of phosphomolybdic acid for potassium permanganate in the pre-treatment gives satisfactory results and does not loosen the sections. We use hydrobromic acid rather than oxalic acid following either potassium permanganate or phosphomolybdic acid, although this step may be entirely omitted with but slight loss of definition after phosphomolybdic acid. Both methods are given in the following outline because the phosphomolybdic acid variation has not been in use a sufficient length of time to afford an accurate comparison with the results obtained by potassium permanganate, particularly on celloidin and frozen sections.

* Received for publication April 8, 1935.

TECHNIQUE

Fixation: Fix tissues in 10 per cent formalin, acetic-Zenker or formol-Zenker.

Embedding Tissues, Cutting and Mounting Sections: Embed in paraffin or celloidin, or cut frozen sections. Paraffin sections are mounted according to the routine method with Mayer's glycerin-albumin and are run through xylol, graded alcohols and distilled water before staining. Celloidin sections may vary in thickness from 4 to 30 microns. The thick sections give a better idea of the density of the fibers in some tumors. They are stained in dishes before mounting. Frozen sections may be stained in dishes before mounting, or mounted on slides and attached with thin celloidin before staining.

Pretreatment: Place the sections in 0.25 per cent potassium permanganate or in 10 per cent phosphomolybdic acid for 1 minute. Rinse in distilled water and place in hydrobromic acid (Merck's concentrated, 34 per cent, 1 part; distilled water, 3 parts) for 1 minute. Hydrobromic acid may be omitted following the use of phosphomolybdic acid.

Sensitization: Wash in tap water, then in distilled water and dip in 1 per cent uranium nitrate (sodium free) for 5 seconds or less.

Impregnation: Wash in distilled water 10 to 20 seconds and place in silver diammino hydroxid (Foot ⁵) for 1 minute:

To 5 cc. of 10.2 per cent silver nitrate add ammonium hydroxid drop by drop until the precipitate which forms is dissolved. Add 5 cc. of 3.1 per cent sodium hydroxid and just dissolve the resulting precipitate with a few drops of ammonium hydroxid. Make the solution up to 50 cc. with distilled water.

Reduction: Dip quickly in 95 per cent alcohol and reduce for 1 minute in the following solution:

Distilled water, 50 cc.; 40 per cent neutral formalin (neutralized with magnesium carbonate), 0.5 cc.; 1 per cent uranium nitrate, 1.5 cc.

Toning: Wash in distilled water and place in 1:500 gold chloride (Merck's reagent) 1 minute. Rinse in distilled water. Place in 5 per cent sodium thiosulphate (hyposulphite) 1 to 2 minutes.

Counterstaining and Mounting: Wash in tap water; counterstain, if desired, with hematoxylin and Van Gieson, or hematoxylin and

eosin; dehydrate in alcohol. Clear in xylol and mount in balsam. The use of ammonia must be avoided in blueing sections after hematoxylin as it dissolves the silver.

The use of distilled water and clean glassware for all solutions is essential. All the solutions may be used repeatedly and kept in Coplin jars for several days. The solutions keep without disintegrating in amber glass-stoppered bottles for an indefinite time.

SUMMARY

Sensitization of sections with uranium nitrate before silver impregnation has reduced the time required for staining reticulum fibers to 10 minutes and has eliminated the necessity of heating the ammoniacal silver. In this way the difficulty of staining sections which are inclined to wash off the slides has been greatly lessened. The substitution of phosphomolybdic acid for potassium permanganate is recommended for such sections as still persist in coming off the slides.

In our experience hydrobromic acid substituted for oxalic acid in the pretreatment, and uranium nitrate substituted for the sodium carbonate of Foot's reducing solution, have improved the staining of argyrophil fibers.

The method presented here is now in routine use in this laboratory. It has proved a time saver and has given even more satisfactory results than the older methods.

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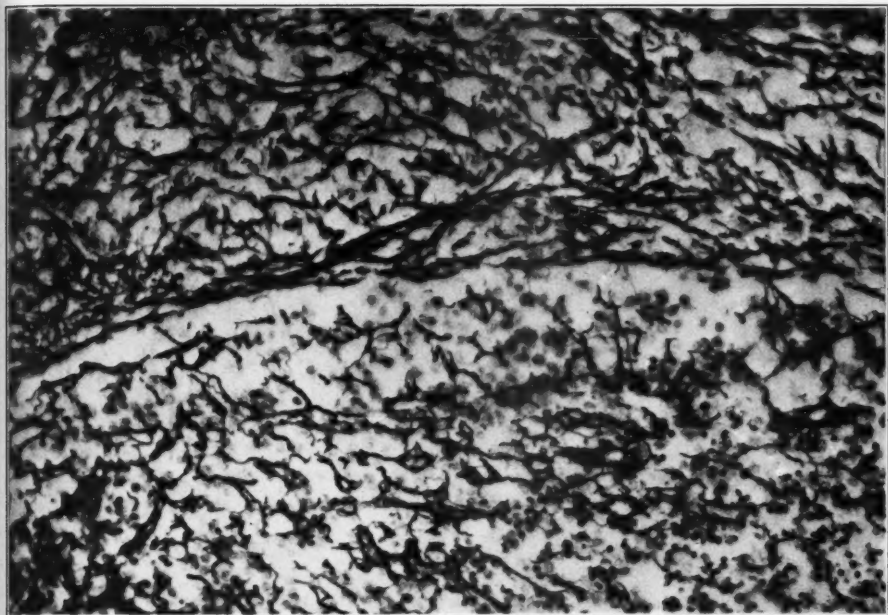
DESCRIPTION OF PLATE

PLATE 117

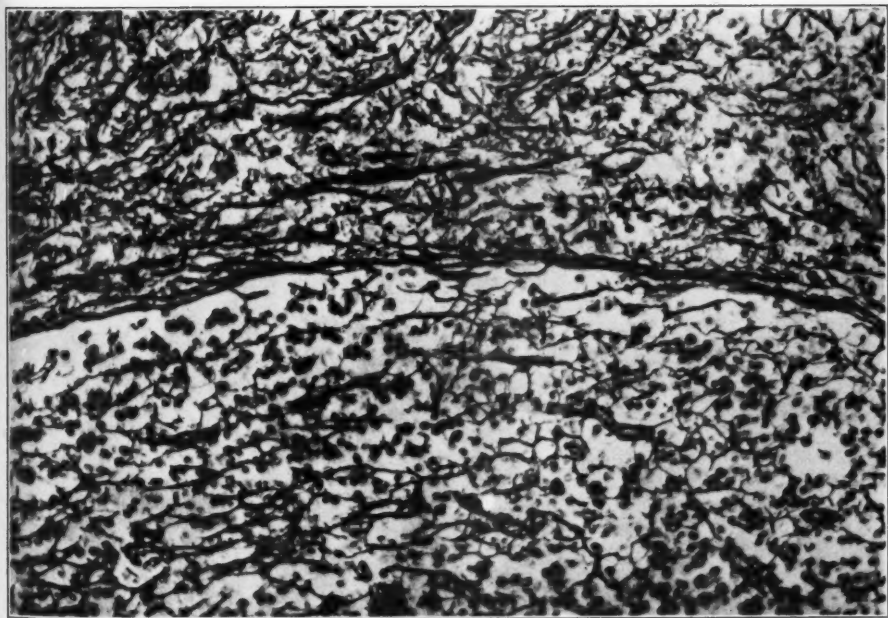
FIG. 1. Silver impregnation of reticulum fibers in a lymph node from a case of reticulum cell leukosarcoma. The slide was stained by the uranium nitrate sensitization method following potassium permanganate and hydrobromic acid. A.M.M. Neg. No. 63006. $\times 215$.

FIG. 2. A slide from the same block stained by the uranium nitrate sensitization method following phosphomolybdic acid, without hydrobromic acid. A.M.M. Neg. No. 63006. $\times 215$.





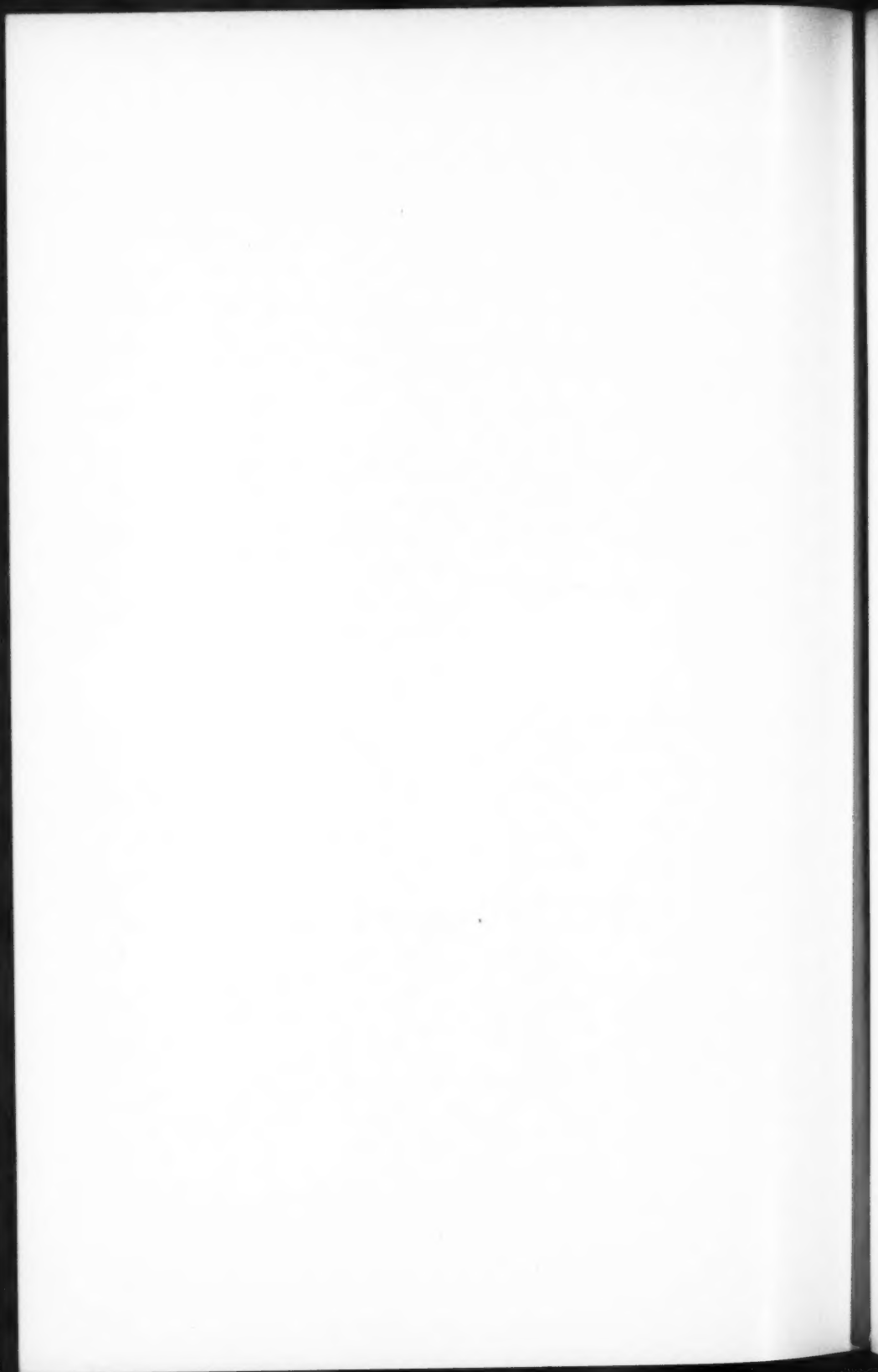
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2

Wilder

Silver Impregnation of Reticulum Fibers



SCIENTIFIC PROCEEDINGS OF THE
THIRTY-FIFTH ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS AND
BACTERIOLOGISTS

HELD AT CORNELL UNIVERSITY
MEDICAL COLLEGE,
NEW YORK CITY,
APRIL 18 AND 19, 1935

THE AMERICAN ASSOCIATION OF PATHOLOGISTS
AND BACTERIOLOGISTS

ABSTRACT OF BUSINESS SESSION

President Boyd in the Chair

The Secretary presented the nomination of the Council for officers as follows:

<i>President</i>	S. BURT WOLBACH
<i>Vice-President</i>	N. CHANDLER FOOT
<i>Treasurer</i>	F. B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	C. V. WELLER
<i>Assistant Treasurer</i>	FREDERIC PARKER, JR.
<i>Assistant Secretary</i>	ALAN R. MORITZ

Voted unanimously to elect those nominated.

Voted to elect the following new members:

William Antopol	Willard S. Hastings
Francis Bayless	Hardy A. Kemp
Allan W. Blair	Gustavus H. Klinck, Jr.
Alfred Blumberg	Enrique Koppisch
Paul Brindley	Leila C. Knox
Osborne A. Brines	Marshall M. Lieber
Edward M. Butt	James B. McNaught
Jefferson H. Clark	John E. McWhorter
Marion C. Corrigan	Perry J. Melnick
Alvin J. Cox, Jr.	W. J. Nungester
Dominic A. DeSanto	Leland W. Parr
Claude E. Dolman	Coleman B. Rabin
Joseph C. Ehrlich	Raymond S. Rosedale
Wiley D. Forbus	Samuel H. Rosen
Thomas Francis, Jr.	Sol R. Rosenthal
William Freeman	Hollis K. Russell
Harold Gordon	Samuel Sanes

Gordon H. Scott
Richard E. Shope
Douglas H. Sprunt

William S. Stanbury
Ernst Witebsky
Krikor Yardumian

It was also voted to reinstate Drs. Donald T. Fraser, DeWayne G. Richey and J. H. Pratt.

Voted to accept with regret the resignations of Drs. B. L. Arms, L. R. Jones, E. O. Jordan, R. H. Major and G. F. Ruediger.

Voted to record with deep regret the deaths of Drs. James Coupal, N. C. Davis, F. S. Jones, Ernest Scott, Theobald Smith, and William H. Welch.

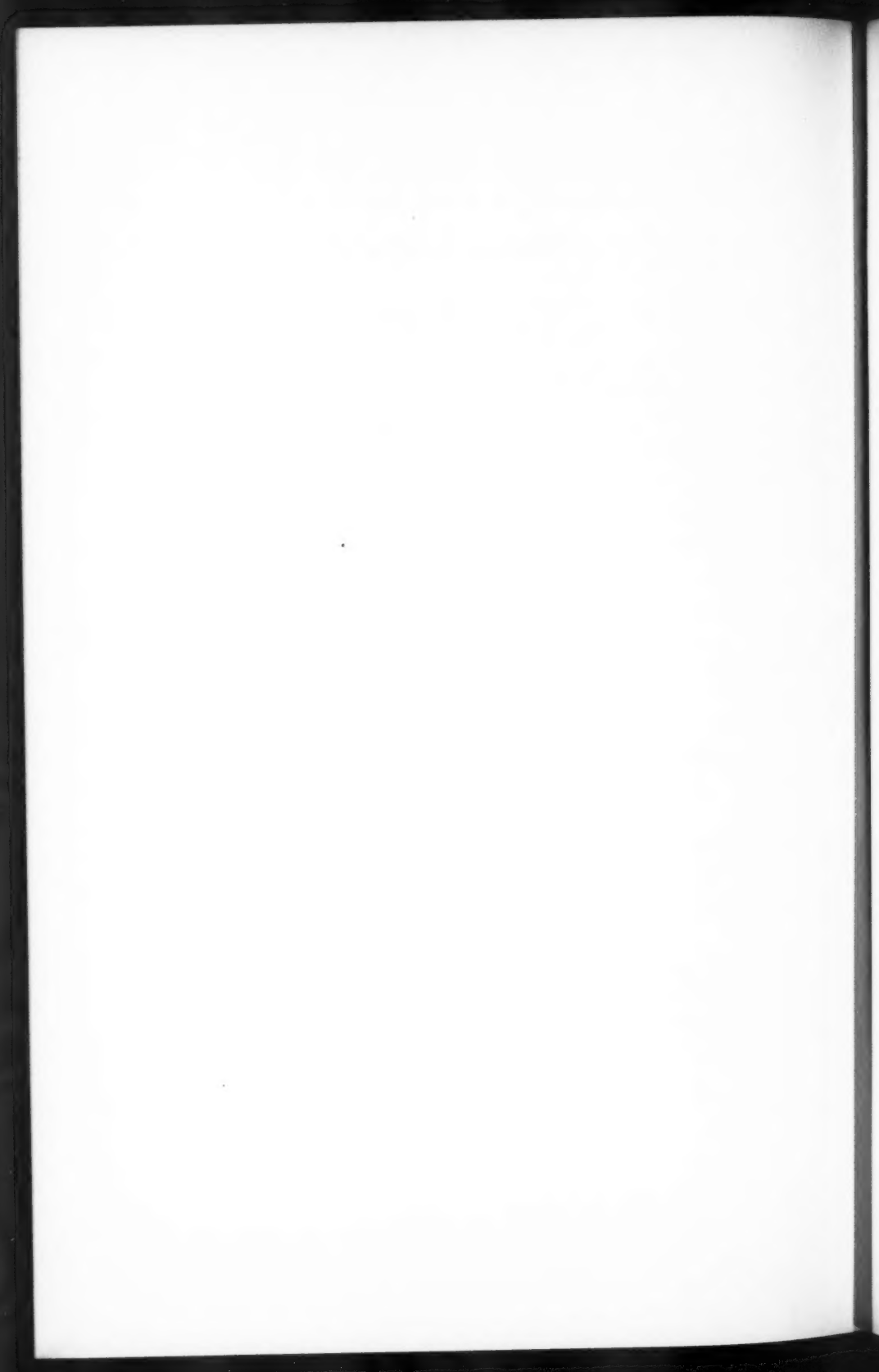
Voted that the Symposium next year be on the subject of Inflammation.

Voted to request Dr. Arnold R. Rich as referee.

Voted to accept the invitation of Drs. F. B. Mallory and Frederic Parker, Jr., to meet in Boston April 9 and 10, 1936.

The Secretary pointed out that through the death of Dr. Theobald Smith a recipient of the Gold Headed Cane of the Association should be selected. It was unanimously voted to select Dr. Frank Burr Mallory for this honor.

Voted to elect Dr. Frederick P. Gay as representative of this Association on the Committee of Type Culture Collection.



AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

A VIRUS DISEASE OF OWLS. R. G. Green, Minneapolis, Minn.

Abstract. From a great horned owl (*Bubo virginianus*) found dead in the wild, a disease was experimentally transmitted to a second great horned owl and to a screech owl (*Otus asio*). Attempts to transmit the disease to a great gray owl (*Scotiopteryx nebulosa*), pigeons and guinea pigs met with failure. The liver and spleen of the owl dying of the natural infection were studded with fine abscesses. Microscopic examination of the organs showed the liver to contain many microscopic abscesses. In the peripheral zone of the abscesses were many intranuclear inclusion bodies involving principally hepatic cells and occasionally an endothelial cell. Similar inclusion bodies were found in the livers of both owls experimentally infected. The distinctive nature of the intranuclear inclusions leaves no doubt that the infection is to be classed as a virus disease. The studies were carried out with Dr. J. E. Shillinger.

Discussion

(Dr. Francis G. Blake, New Haven.) Have you any indication as to the natural method of transmission of the virus?

(Dr. Thomas M. Rivers, New York City.) I should like to suggest, inasmuch as there is a virus disease of parakeets in Brazil exactly like that described by Dr. Green, that he attempt to transmit it to parakeets. Might you not find them a susceptible host?

(Dr. Green, closing.) Unfortunately as this virus was lost we were unable to carry out extensive studies on transmission, and we have no indication as yet as to how the disease is transmitted.

I appreciate Dr. Rivers' suggestion. We are carrying out extensive studies of wild life, and owls are continuously under observation. We have maintained a stock of horned owls now for 2 years, hoping to meet this virus again.

A FILTRABLE VIRUS FROM WHITE MICE. Erich Traub (by invitation), Princeton, N. J.

Abstract. If mice from our apparently healthy breeding colony are given an intracerebral inoculation of sterile bouillon a small proportion die after showing characteristic symptoms. Death is due to a filtrable virus which can be transmitted in series by intracerebral inoculation in mice and guinea pigs. The latter can also be infected by material injected subcutaneously or intranasally and are the best animals to use in detecting the virus. Rats inoculated intracerebrally develop the disease.

The outstanding pathological changes in experimentally infected mice are a marked meningitis and infiltration of the chorioid plexus with mononuclear cells, mostly lymphocytes. The virus is present in the blood and urine of diseased mice as well as in the central nervous system.

The pathological changes in guinea pigs are similar to those in mice, except that cellular infiltration of the meninges and chorioid plexus is much less marked than in mice. Acidophilic intranuclear inclusions were found in guinea pigs in meningeal cells, in mononuclear cells present along the meninges, in adventitial cells of meningeal vessels, in glia cells near the pia mater and, very rarely, in epithelial cells of the chorioid plexus. Pneumonia of the virus type is frequent in guinea pigs inoculated intranasally, intracerebrally, or subcutaneously with virus.

Cross-neutralization experiments show that our virus is serologically identical with the virus of lymphocytic choriomeningitis described by Armstrong (*Pub. Health Rep.*, 1934, 49, 1019). It has further been found that the blood serum of the man who takes care of our breeding mice neutralizes the virus recovered from the stock.

Discussion

(Dr. Thomas M. Rivers, New York City.) I think Dr. Traub's virus is of interest for two reasons: first, individuals who are working with viruses in mice might mistake this new virus for the active agents with which they are working. Second, Dr. Traub's virus is pathogenic for man. Dr. Scott and I saw two men who had the clinical picture of meningitis — stiff neck, fever, Kernig sign, and marked increase of cells in the spinal fluid, practically all of which were mononuclears. From the spinal fluid of these two patients we obtained a virus identical with that of Dr. Traub. The virus is not in our stock mice. Furthermore, the patients had no neutralizing antibodies for this virus at the onset of illness, but developed them during convalescence.

(Dr. Ralph D. Lillie, Washington.) The pathological response in Dr. Traub's mice apparently differs in some respects from that studied by Armstrong and myself in Washington in that we have seen less tendency to invasion of the central nervous system and the reaction appears to me to have been less marked in the choroid plexi than would be indicated from Dr. Traub's description. The meningeal reaction has been very similar in nature, however.

(Dr. Maurice Brodie, New York City.) I should like to ask Dr. Traub what concentration of the emulsion he used in these mice, because we have picked up in mice a spontaneous virus which seems to be a little different from his; that is, the incubation period is 2 to 4 days when we use a 10 per cent suspension. If we use weaker dilutions, 1:100 or 1:150 suspensions, the incubation period becomes longer. The disease is rather more acute; convulsions occur spontaneously and there is much more tremor. The pathology is a little different, inasmuch as meningeal involvement is not as marked, and there is but little choroid involvement, more hyperemia, and focal areas of necrosis in the posterior horns are found. I wonder whether the greater reaction we get can be due to greater concentration and whether we are dealing with the same thing. We have not succeeded in transferring it to guinea pigs, but we have given it to rabbits.

(Dr. Traub, closing.) In reply to Dr. Lillie, the necrosis of nerve cells does not occur in all mice and the number of necrotic cells is fairly small, but we considered the necrosis was definite because we got neuronophagia and all the features which are essentially connected with necrosis.

I should like to answer Dr. Brodie that it does not make much difference what the concentration of virus is that we inoculate into mice. The incubation period is not shorter than 5 days.

THE VIRUS OF LYMPHOGRANULOMA INGUINALE. Rigney D'Aunoy, Emmerich von Haam and (by invitation) Louis Lichtenstein, New Orleans, La.

Abstract. Although lymphogranuloma inguinale appears to be a rather common disease in the United States and numerous case reports have been published from various parts of this country, comparatively little has been done by American authors in systematically studying its causal virus. In the course of our clinical study of this disease in its various manifestations in New Orleans, 160 cases were observed over a period of 6 months. From 7 of these cases the filterable virus etiologically associated with the disease was isolated and kept viable and infectious through repeated animal passage. The physical and biological characteristics of these local virus strains were studied and compared with similar findings of European investigators. This was accomplished by means of: (1) animal passage of the virus through homologous and heterologous species of experimental animals, using various routes of inoculation; (2) consideration of the antigenic value of fresh and desiccated organ emulsions of infected animals for the diagnostic Frei reaction; (3) cultivation of the virus; (4) animal protection experiments, and (5) histopathological studies.

Discussion

(Dr. Arthur William Grace, New York City.) I have enjoyed the presentation of this paper very much. Dr. von Haam has covered a great deal of work in a comparatively short time and the results he has found have been very much in line with what we have found in the work on lymphogranuloma inguinale. There is one point on which we differ: he spoke of the autosterilization of the virus. We have not detected that at all. We have now passed our strain in 12 months through 70 to 75 generations of white mice and have not detected any trace of autosterilization whatever. The virus is just as strong now as during any passage en route. Whether we are dealing with a stronger virus I do not know; I rather feel it is, because our results from the beginning showed a shorter period of incubation in mice than has been reported by any other worker. Another point that we have been working on here is the use of the infected brain as an antigen. We have obtained no non-specific reactions, and the material obtained from the mouse brain has produced as strong a Frei reaction as can be obtained with the most potent human lymphogranulomatous pus. The lack of good Frei antigen has hampered the diagnosis of the disease for a long time, but now that the disease can be transmitted so readily to mice and the infected mice brains do not contain a suspicion of any other venereal disease, mouse brain antigen is very suitable for distribution. Lederle has taken the mouse strain of virus we are using and is now putting up the material commercially, so that I suspect in a short time lymphogranuloma inguinale mouse brain antigen will be available in an unlimited supply for the diagnosis of the disease, and then we shall see what a large number of cases there must be scattered throughout the country. We shall probably find there are more rectal than inguinal cases, because the rectal cases are with us all the time. In the inguinal cases the patients get better and pass out of sight, and I think the rectal surgeons will find the use of lymphogranulomatous mouse brain very valuable for the diagnosis of a number of obscure cases of benign inflammatory rectal disease. It is due to the efforts of Dr. von Haam and his co-workers and the people in France and Scandinavia that we have been able to replace the very unesthetic human lymphogranu-

lomatous antigen by the animal antigens which are just as specific and always available in large quantity.

(Dr. Maurice Brodie, New York City.) I should like to ask whether or not all of Dr. von Haam's animals came down with symptoms, or whether a good many did not, and whether he transferred the virus after 2 to 4 weeks in the manner Levaditi has. We have tried 6 or 7 strains by that method but have not had the success which he had. In 2 we got the transfer to mice, and 1 to guinea pigs, and none of the animals came down with symptoms. Two to 4 weeks after the injection some of the mice would die, and so we tried transferring it at 2 week intervals and tested the material in patients, and we found the reactions in the skin got weaker with successive transfers until it lost all its strength, usually in 3 or 4 passages, and so I wondered what kind of reaction he had in the majority of his animals.

(Dr. Enrique E. Ecker, Cleveland.) I should like to ask Dr. von Haam if any attempts have been made to use the culture instead of the usual Frei antigen, and whether the culture or brain emulsion will give skin reaction test in high dilutions. We got a reaction with the Frei antigen in 1 case using a dilution of 1:20,000. However, reactions occur commonly with dilutions of 1:5000. A positive test has been obtained as long as 39 years after the infection.

(Dr. Morris I. Rakieten, New Haven.) I should like to ask Dr. von Haam about the cloudiness in the medium. Does he interpret that as an actual growth, a proliferation of the virus in an artificial medium?

(Dr. William Boyd, Winnipeg.) How many cases of the disease due to this virus have been reported in Canada?

(Miss Suskind, New York City.) I should like to ask Dr. von Haam, in relation to the cultural activity of the virus, whether he has been able to stain the fluid and demonstrate what might be described as filterable virus bodies.

(Dr. von Haam, closing.) In regard to autosterilization, we have observed it twice in our 7 strains. Two of our strains died out through continual passage through mice. I believe Dr. Grace has an especially strong virus strain, because of the incubation time and because he has successfully transmitted it so long.

As to the distribution of the disease in Canada, there is only one report in a French Canadian journal which unfortunately was not available to me. That reported 3 cases in the vicinity of Quebec.

I am unable to answer the question as to the dilution of the virus and of the brain emulsion as we did not work with this problem. The literature reports usually the virus cannot be diluted very much and negative results in a dilution of as high as 1:10,000 are reported.

In regard to Dr. Brodie's remarks as to symptoms, we keep the virus now going by routinely transmitting the virus every 2 weeks, and most of our mice do not show symptoms by this time. The incubation time in our strains for the appearance of symptoms is 3 to 4 weeks, but characteristic histological changes can be found in mice as early as 5 to 6 days after the inoculation.

In reply to Miss Suskind's remarks about staining the virus, we did not stain the virus. However, Tamura stained the fluid with virus stain, and he found bodies which he believes to be virus.

I do not know that the cloudiness actually represents the virus, but it does not appear in the control animals. The cloudiness can be transmitted from tube to tube and, as Tamura has shown, the virus can be centrifuged with about 6000 revolutions per minute down in the deeper layers.

THE PRESENT STATUS OF THE ANTIGENIC ANALYSIS OF THE ELEMENTARY BODIES OF VACCINIA. James Craigie (by invitation), Toronto, Canada.

Abstract. It has been reported that two types of reaction may occur when suitably prepared extracts of vaccinia-infected tissue are incubated with rabbit antivaccinia serum (*Brit. J. Exper. Path.*, 1932, 13, 259). A precipitin reaction takes place with "free" antigen in the extract and, in addition, any elementary bodies which may be present are agglutinated. More recently it has been shown that rabbit antivaccinia serum contains two types of agglutinin for the elementary bodies of vaccinia (*Brit. J. Exper. Path.*, 1934, 15, 390). The corresponding agglutinogens differ markedly in their stability. The L or labile antigen is inactivated by heat at 56° C., by excess of formalin, by the photodynamic effect of methylene blue, and by other manipulations which render the elementary bodies non-infective. The S or stable antigen, on the other hand, withstands a temperature of 100° C. This paper presents further observations on these antigens.

It may be shown by means of suitable precipitin tests on the wash fluids that in a freshly prepared and washed elementary body suspension all the antigen is fixed to the surface of the elementary bodies. On storage, however, particularly if distilled water saturated with ether is employed as the suspending fluid, a portion of the antigens becomes dissociated from the elementary bodies. These dissociated antigens are demonstrable by subjecting the suspending fluid, after removal of the elementary bodies, to precipitin tests. The use of serums containing either L or S antibody alone has shown that both the corresponding antigens dissociate. Dissociated L antigen is as labile as L antigen fixed to the elementary bodies. Repeated but decreasing yields of "free" antigen may be obtained and the elementary bodies remain agglutinable and infective. However, the appearance of fine particles in the suspension, and a decrease in infectivity, suggest the possibility of disintegration of some of the elementary bodies of lesser resistance.

Pure L and S precipitin serums, controlled by appropriate tests with elementary bodies and antigen dissociated from them, have been used to determine the nature of the antigens found in Seitz filtrates of vaccinia-infected skin. Such filtrates, free from elementary bodies, contain both L and S antigen. The conditions under which these filtrates are prepared make it seem highly probable that the antigens exist in the "free" state in vaccinia-infected skin. Both L and S antigens have been found in the vaccinia-infected skin of the calf and guinea pig, as well as in that of the rabbit.

It has been suggested that the "antigens" involved in the *in vitro* reactions of some viruses are of extrinsic origin, arising from host substances as a result of the infective process. No observations have been recorded which are inconsistent with the view that the L and S antigens of vaccinia are intrinsic products of the elementary bodies, analogous to bacterial antigens, which may dissociate *in vivo* as well as *in vitro*. The comparatively large quantities of L and S antigen which seem to be present in the "free" state in vaccinia-infected skin, taken in combination with other observations, would seem to suggest that vaccine virus particles may vary greatly in their resistance to disintegration. Elementary body suspensions prepared by the usual methods may possibly represent only the more stable forms of the virus with spore-like qualities of high resistance to an adverse environment. Actively proliferating intracellular virus, not neces-

sarily differing in morphology from elementary bodies, may be much less viable if separated from living cells.

The possibility that vaccine virus particles of varying viability when separated from the cell may exist in living tissue, but that only the more resistant forms can be manipulated *in vitro*, should be borne in mind in interpreting failures to demonstrate inactivation of the virus by immune serum *in vitro*.

Discussion

(Dr. Frederick P. Gay, New York City.) I should like to ask which of these two antigens gave rise to immunity, or whether they both did.

(Dr. Claus W. Jungeblut, New York City.) I did not quite hear what Dr. Craigie said about the neutralization experiments, but I would like to know which of the two serums, the S or the L type, was more active in neutralizing the virus *in vitro*.

(Dr. Craigie, closing.) I am afraid that I have been asked two questions which I cannot answer. I do not know which of the two types of antibody is more active in neutralizing the virus or whether either of them is active in this respect. As regards immunity, I think that I can say this — there is no evidence which would indicate that the S antigen and corresponding antibody play any significant part in immunity. The L antigen may be involved, but I am not certain. It is true that vaccinia elementary bodies inactivated without loss of the L antigen appear to produce a much greater degree of immunity than elementary bodies inactivated with concomitant loss of this antigen. This, however, does not necessarily mean that the L antigen is involved in immunity. There may be some other labile antigen which we cannot demonstrate *in vitro*.

RESPONSE OF RABBITS TO FORMOLIZED WASHED ELEMENTARY BODIES OF VACCINIA AND TO VIRUS-FREE FILTRATES OF DERMAL VACCINE VIRUS. Robert F. Parker (by invitation), New York City.

Abstract. It has been shown by Craigie that injections in rabbits of inactive elementary bodies of vaccinia cause the appearance of agglutinins, precipitins, and complement-fixing antibodies. Washed suspensions of elementary bodies were prepared by Craigie's technic, which were almost entirely free of other particulate material. The virus was inactivated by means of 0.3 per cent formaldehyde, large amounts being tested for the presence of active virus by serial testicular passage in rabbits. Extracts of dermal virus containing the soluble antigens of vaccinia were obtained and freed of virus by passage through collodion membranes. These were similarly tested for the presence of active virus.

Rabbits kept under isolation precautions were injected twice weekly for 6 weeks with increasing amounts of these materials, a total of 18 cc. being inoculated. Serum was taken and the rabbits were tested for immunity by means of dermal, intradermal and testicular inoculations of virus.

After immunization, agglutinins for elementary bodies and precipitins against the soluble antigens were present in moderately high titer, while virus-neutralizing antibodies were present in small amount. The rabbits were moderately or completely refractory to infection with a weak culture virus, but only partially immune to a stronger testicular strain.

A striking phenomenon was noted, *i.e.* animals immunized with inactive elementary bodies, and almost entirely refractory to infection with culture virus, were still moderately susceptible to a later inoculation of potent testicular virus.

Discussion

(Dr. James Craigie, Toronto.) Dr. Parker's paper raises some interesting points. I might say in the first place that his results are in agreement with those obtained by us in our earlier attempts to produce immunity with inactivated vaccinia elementary bodies. The observations made during the course of this preliminary work were one of the factors in determining a search for a labile type of antigen in the elementary bodies of vaccinia. I should emphasize that Dr. Parker in his formalized washed elementary bodies has used an amount of formalin which in my experience will completely inactivate the L antigen as well as abolish the infectivity of the elementary bodies. More recently I have been attempting to inactivate the elementary bodies of vaccinia with the least possible amount of formalin and this has been facilitated by the use of pure L sera. If one succeeds in inactivating the elementary bodies, leaving the labile antigen more or less intact, then one can produce a much more definite degree of resistance to skin inoculation of vaccine virus. This resistance may be measured in two ways, and the results may differ somewhat according to the method of the test, *i.e.* whether skin scarification or intradermal injection is employed. One manifestation is a definite resistance to inoculation of the virus, and the other is a very definite acceleration of the reaction. One may in rabbits produce quite marked resistance to inoculation with inactivated elementary bodies, but this immunity does not compare in its duration to immunity developed after inoculation of living elementary bodies. I think that the present interest of this type of investigation lies not so much in the attempt to produce immunity by inactivated vaccinia virus, but in the investigation of the mechanism of immunity to this virus.

(Dr. Harry S. Eagle, Philadelphia.) I think it is interesting that an antigen was found that would pass through a collodion membrane. It indicates that particles of small molecular size may be antigenic. I should like to ask Dr. Parker if he has any data on the permeability of the filter he used.

(Dr. Parker, closing.) In reply to Dr. Eagle, the membranes used were prepared by Bauer in the Yellow Fever Laboratory and the average diameter of the pores is 103 millimicra.

DEVELOPMENT OF IMMUNITY TO FOX ENCEPHALITIS. R. G. Green, Minneapolis, Minn.

Abstract. Serum of animals recovered from fox encephalitis contains antiviral and this can be increased by hyperimmunization. The maximum antiviral content is developed after more than 1 year's continuous injection of virus. The injection of serum-virus mixtures into normal foxes leads to death of the more susceptible animals after 30 days, when the serum has been eliminated. Development of acquired immunity to fox encephalitis seems to require several weeks for the most susceptible individuals. Recovery from fox encephalitis appears not to depend upon acquired immunity, but upon the extent of natural immunity at the onset of the disease. The studies were carried out with Dr. J. E. Shillinger.

Discussion

(Dr. E. Watson, Ottawa.) I should like to ask Dr. Green if he has done any cross-immunization tests with fox encephalitis and canine distemper virus and, if so, has he found that one has any neutralizing effect on the other?

We have done a few experiments along those lines, and there is a tentative suggestion in them that there is some protection offered by canine distemper virus against fox encephalitis virus, and *vice versa*.

(Dr. Claus W. Jungeblut, New York City.) I should like to ask Dr. Green if he has formulated any ideas about the mechanism of the natural immunity he spoke of in his paper. Is it acquired by previous infection?

(Dr. Green, closing.) In reply to Dr. Watson's question, I should like to state that our extensive investigations have been confined largely to a particular strain of virus, the Fromm strain, and we have been unable to discover any relation whatsoever with the canine distemper virus.

Dr. Jungeblut's question is one with which we have concerned ourselves. In our experimental ranch we maintained strict quarantine for a number of years. The foxes on the ranch were principally red fox pups dug out of their dens in the wild and transported by litters to the ranch. We are quite positive that the immunity shown by foxes from this group was not acquired by contact with the virus. We have studied the breeding records of fox groups being put into an epidemic area each fall. It was a very striking observation that each year the progeny of certain pairs of foxes would always die of fox encephalitis, while the progeny of other pairs never died of the disease, although all were exposed together. This definitely appears to be evidence of inherited natural immunity.

STUDIES ON THE MECHANISM OF IMMUNITY IN CERTAIN VIRUS DISEASES. Albert B. Sabin (by invitation), New York City.

Abstract. Since the protective substance in immune, antiviral serum is capable of exerting its effect *in vitro* in a simple system consisting of minced susceptible tissue, immune serum and virus, the rôle and fate of each of the constituents in the consummation of the immune process were analyzed. With the aid of an ultracentrifuge (14,000 r.p.m.), which rendered possible the complete sedimentation and quantitative recovery of vaccinia, pseudorabies and B virus, it was found that the protective substance in antiviral serums neither combined with nor inactivated these viruses even after prolonged periods of incubation. In cultures containing susceptible tissue, immune serum and virus, the protective effect was exerted without any demonstrable direct effect upon the virus; the protective substance apparently acted upon the tissue by rendering it refractory to "infection," and unsuitable for the multiplication of the virus. By treating normal susceptible tissue with antiviral serum and then separating the tissue from the serum, it was possible to show that it had been rendered refractory to infection even though it was surrounded by and had "fixed" active virus. It was also found that the protective substance is fixed by the tissue and that both the fixation and the effect are reversible by washing. Experiments with leukocytes revealed that while they take up or fix virus, they become highly infectious thereby and thus play no part in preventing infection; no opsonic effect of immune serum on virus was demonstrable.

The varying protective power of antiviral serum *in vivo* in different tissues of the same species and in the same tissues of different species was studied: it was found that this phenomenon could be accounted for neither on the basis of any direct action of the immune serum upon the virus *in vitro* or *in vivo*, nor on the basis of the varying ability of the tissues to neutralize small amounts of virus. It appeared rather that different tissues require different amounts of protective substance for protection against the same amount of virus and that certain

other, as yet obscure, factors are involved. These studies suggest that the virus itself may not be the direct antigenic stimulus, but that some substance upon which it acts and which becomes antigenic when liberated from infected cells may be the factor responsible for the formation of the immune protective substance.

Discussion

(Dr. R. G. Green, Minneapolis.) I shall look forward with a great deal of pleasure to seeing the details of this paper, as one of the conclusions does not appear to correspond with our experience. In one of our experiments 25 foxes were given an injection of serum to produce a passive immunity. Of the first 10 foxes inoculated with virus 10 days later, 8 died with typical encephalitis. The same amount of serum would have given protection for 30 days if the serum had been mixed with the virus before injection. This would make it appear that the effect of the serum was on the virus rather than on the tissue. We also have seen the variation of immunity apparently dependent upon the tissues involved. In our preliminary investigations on virus neutralization all inoculations were made by cisterna puncture. In these experimental animals we observed no delayed infections. Among some 2000 ranch foxes inoculated with similar mixtures by intramuscular injection, the disease regularly appeared some 30 days after inoculation as delayed infections. Following this the delayed infections were observed in experimental animals when the serum-virus mixture was injected intramuscularly.

(Dr. Joseph D. Aronson, Philadelphia.) I should like to ask what tissues are especially susceptible to the virus, whether you can produce selective immunity in such tissues, and lastly, whether the serum can be removed from the tissues so as to again make the tissue susceptible to infection with the virus.

(Dr. L. Dienes, Boston.) In connection with the observation which I reported yesterday, I should like to mention that in guinea pigs the development of immunity response after infection with vaccinia virus is about the same as after injection of eggwhite. If a large area of the skin is infected, antibodies appear in the blood first after the 12th day. However, the skin lesions begin to heal on the 5th day. At about the same time the non-infected part of the skin begins to give slight hypersensitive reactions. These reactions are rather strong on the 8th day. These skin reactions correspond to the tuberculin reaction and to the slight skin reaction which we observed between the 4th and 7th days after eggwhite injection. They are characterized by delayed appearance and a mainly mononuclear infiltration of the tissues. This type of skin sensitiveness corresponds to the active immunization of the tissues, and circulating antibodies never produce a similar condition. In guinea pigs the infection of the skin heals in the active stage of the immunization process before the appearance of antibodies in the circulation. We do not know the relation of antibodies to this condition; it certainly cannot be reproduced by passive immunization. The study of this condition may be as necessary for the understanding of healing and immunity as the study of antibodies.

(Dr. Francis G. Blake, New Haven.) It would appear to me that the conclusions from these experiments are consistent with some of the observed phenomena met in disease. I shall cite only two: first, the observed fact that in the prophylactic use of convalescent serum in measles the serum must be used before the virus enters the blood, if prevention is to be obtained; second, the ob-

served fact that in herpes febrilis the disease may frequently recur in an individual in spite of the fact that he has neutralizing antibodies in his blood. I should like to ask Dr. Sabin if these two observed facts, which are illustrative of others, seem to him consistent with his conception.

(Dr. Sabin, closing.) In reply to Dr. Green, I must say that I have made a careful study of many of his interesting experiments with the virus of fox encephalitis and found them strikingly in accord with the conception that the protective action of antiviral serum is not the result of any direct action on the virus. In one particular type of experiment, in which Dr. Green injected mixtures of serum and virus subcutaneously, the animals appeared to be completely protected for a long period (a month or more), and then suddenly would develop the disease. During this entire period apparently nothing happened to damage the virus, and when the passive immunity conferred by the serum had worn off the animal developed the typical disease. Somewhat similar experiences have been encountered in dog distemper work when immune serum and virus were used for immunization. The fact that Dr. Green observed no delayed infection when he used the cisternal route may mean that the virus does not survive as long there as in the subcutaneous tissue. The spontaneous deterioration of viruses which may vary in different tissues may play an important part in determining the ultimate infectivity of a serum-virus mixture.

In response to Dr. Aronson, the susceptibility of different tissues probably varies with individual viruses. I cannot answer the second question, but the third one regarding the possibility of removing the serum from immune tissue and thus rendering it more susceptible to infection can be answered in the affirmative. Rivers and co-workers have shown that the excised cornea from a rabbit immune to vaccinia may occasionally be deprived of its resistance to infection *in vitro* by washing, and Andrewes found that testes from actively immune herpes and virus III rabbits were rendered highly susceptible to *in vitro* infection by washing. In my own work it was shown that while normal tissue could be rendered refractory to infection by exposure to immune serum, it would lose this refractory state upon repeated washing.

The observations on the prophylactic effect of measles convalescent serum chiefly before the generalization of the virus and on the occurrence of herpes febrilis in individuals with antibodies for the virus in their blood, which Dr. Blake has mentioned, would not appear to be inconsistent with the conception that the antibodies in immune serum do not act directly upon the virus. In the case of measles, it is clear that the cells must be, and perhaps are, protected before the virus reaches them to avoid the systemic disease, while in the case of herpes febrilis the present conception explains at least why a virus survives and may be carried by an immune host in the presence of immune bodies, which do not "neutralize" it even when it is unprotected by a probable intracellular habitat.

EVIDENCE OF ACQUIRED IMMUNITY FROM PLANT VIRUS DISEASES. L. O. Kunkel
(by invitation), Princeton, N. J.

Abstract. Tobacco seedlings regularly recover from, and are thereafter immune to, the disease caused by the tobacco-ringspot virus. Recovered plants, although indistinguishable from plants that have never had the disease, retain the ring-spot virus indefinitely. Plants propagated from cuttings of recovered plants are likewise immune, but plants grown from seeds are susceptible.

More than fifty different strains of the tobacco-mosaic virus have been isolated. The several strains cause diseases that differ in severity, from some that are extremely mild to others that are lethal for young plants. Seedlings infected by a mild strain of virus become immune from severe strains. They are not, however, protected against tobacco ringspot, cucumber mosaic, or any plant virus disease unrelated to tobacco mosaic. The protection test furnishes an easy means of determining whether or not any new disease belongs in the tobacco mosaic group. Similar tests with the tobacco-ringspot virus and with viruses causing other diseases indicate that the immune reaction is specific. Results obtained from protection tests have been confirmed by serological studies.

Discussion

(Dr. Everett G. D. Murray, Montreal.) I should like to ask Dr. Kunkel if the susceptible plant is budded or grafted on to an immune or recovered plant whether there is any protection or resistance transferred to the bud or graft.

(Dr. Kunkel.) No, the bud or graft does not acquire immunity without an attack of the disease.

(Dr. James Ewing, New York City.) I should like to ask Dr. Kunkel to tell us a little more about the conditions of survival of the virus in plants that do not show the symptoms of the disease.

(Dr. Augustus B. Wadsworth, Albany.) I should like to know how long the virus persists in the transplants of the cuttings. I assume that it continues.

(Miss Suskind, New York City.) Before I ask my question I must plead ignorance of botanical technique. I would like to know, however, if it is possible to macerate recovered plant tissue and demonstrate tissue immune bodies, either by prophylactically treating well plants with that macerated plant extract and subsequently attempting to infect them, or by taking plants already infected and treating them with the macerated extract.

(Dr. Stuart Mudd, Philadelphia.) Is it possible to obtain passive immunity by transfer of sap or other plant juices?

(Miss Feig, New York City.) There appear to be about fifty different types of plant virus disease. How do you tell the different types apart?

(Dr. Arthur William Grace, New York City.) I should like to ask whether the seeds of plants that are non-immune come from plants that were previously infected, or whether they are seeds from other plants.

(Dr. Kunkel, closing.) Regarding the seeds, to answer the last question first, the viruses do not usually pass through the seeds. There are a few exceptional cases, like the virus disease of the bean, which is transmitted through the seeds, but most viruses are not transmitted through the seeds of plants. Plants grown from seeds of diseased plants are quite healthy.

Regarding how long the virus persists in cuttings, we have some cuttings that have been grown in successive generations for over a period of 4 years, and the virus is there just the same as when the plant first recovered.

In reply to Miss Feig, as to how we recognize these different strains, we recognize them by the symptoms they produce in the plants.

In reply to Miss Suskind, we have no evidence whatever of immune bodies. We know nothing of the mechanism by which the plants are protected. You must remember there is no blood stream to play with.

(Dr. William Boyd, Winnipeg.) Dr. Mudd asked whether there was a sap stream to play with.

(Dr. Kunkel.) Yes, there is a sap stream, but it is not comparable to the blood stream; it is not circulating.

THE DIFFERENTIATION OF PLANT VIRUSES BY THE SERUM-PRECIPTIN REACTION. Helen Purdy Beale (by invitation), New York City.

Abstract. If the expressed juice of a plant affected with a filterable virus disease is used to hyperimmunize rabbits, the resultant antiserum contains precipitins, neutralizing and alexin-fixing antibodies.* The serum reactions are specific for the homologous virus extract employed as antigen, and the precipitin reaction may be used to great advantage in the recognition of new host plants, in the detection of "carriers" and in the differentiation and classification of viruses. Plant viruses which are regarded as distinct on the basis of symptomatology, host range, properties, methods of transmission and plant immunity tests, are likewise distinguishable by the serum reactions. Qualitative and quantitative investigations of the serum-precipitin reaction indicate a close association between active virus and antigen.

Discussion

(Dr. Arthur William Grace, New York City.) I should like to ask Dr. Beale in what animal the serum was produced. That was not quite clear to me from what she had to say.

(Miss Suskind, New York City.) I should like to know how purification of the virus was carried out. Was it by filtration only or were chemical methods also employed?

(Dr. Beale, closing.) In answer to the first question, the rabbit was used. I also neglected to say there are neutralizing bodies in the antiserum.

In answer to Miss Suskind, the methods of purification are chemical and also by the use of the Seitz filter.

TITRATION OF ANTIBODIES IN SERUMS OF PERSONS RECEIVING ANTIRABIC TREATMENT. Leslie T. Webster and (by invitation) J. R. Dawson, Jr., New York City.

Abstract. A mouse protection test has been developed for titrating antirabic substances in serums. Street rabies virus passed intracerebrally in Swiss mice 3 to 15 times is diluted, mixed with the undiluted test serum and injected intracerebrally into Swiss mice. Mice receiving mixtures of normal sera plus final dilutions of virus as high as 10^{-6} succumb to rabies after consistently uniform incubation periods. Repeated tests with the same and different normal sera and with the same and four different strains of virus have given uniform results except that early passage virus results in relatively long incubation periods in injected mice.

The protection test is being used to measure the development of antirabic substances in the blood of humans following antirabic treatment. Serum from an individual 2 and 15 years after treatment with "T," "T," and "L" vaccines protected completely against 100 lethal doses of 4 strains of mouse passage virus. This serum diluted 1:50 showed complete protection against 10 lethal doses. Of serums from three individuals tested on the 1st, 8th, 14th, 21st and 30th days

* Beale, Helen Purdy. The serum reactions as an aid in the study of filterable viruses of plants. *Contrib. Boyce Thompson Inst.*, 1934, 6, 407-435.

of treatment with "N" Semple vaccine, one of the 30 day serums showed slight protection, while the remainder were practically negative. Serums from three individuals 8 months after a 21 dose treatment with the same "N" vaccine and from one individual 8 months after a 28 dose treatment with "N" vaccine showed no protection. On the other hand, three pools of 5, 5, and 4 serums from fourteen persons on the 14th day of treatment with "G" Semple vaccine protected against 10 lethal doses, and serums from five of these same persons tested after 37 days, and five tested after 62 days protected fully against 100 lethal doses of virus. And finally, serum from an individual given "H" vaccine 13 and 5 years ago protected against 100 lethal doses.

The "N" and "G" vaccines injected into Swiss mice failed to incite rabies; the "H" vaccine so treated did bring the animals down with rabies.

Discussion

(Dr. Ralph D. Lillie, Washington.) May I ask what type of antirabic vaccines was used in these cases, — whether phenol-killed or the old Pasteur vaccine?

(Dr. Webster, closing.) All vaccines thus far tested have been of the phenol-killed Semple type, with one exception. In this case the "H" vaccine was dried and diluted.

ON THE PROBLEM OF IMMUNIZATION AGAINST POLIOMYELITIS. E. W. Schultz and (by invitation) L. P. Gebhardt, Stanford University, Calif.

Abstract. Passive immunization of monkeys with large doses of a high titer immune serum provides some protection against subsequent intracranial or intranasal inoculations with virus. For protection against a given dose of virus a disproportionately high antibody titer is necessary. A relatively low protection is afforded against virus administered by the intranasal route. This may be explained by the fact that the olfactory nerve, the usual portal of entrance, is very accessible to the virus and cannot be effectively guarded by immune substances in the blood plasma.

Results obtained following artificial active immunization with variously prepared vaccines indicate that it is much easier to stimulate the formation of antibodies than it is to alter the susceptibility of neurons. Since a true acquired active immunity to poliomyelitis is basically a cellular rather than a humoral phenomenon, the criterion of successful active immunization must be an unquestionable increase in tissue resistance, rather than the appearance of antibodies. A well defined increase in neural resistance seems difficult to obtain even when living virus suspensions are used as vaccines.

PATHOLOGICAL AND IMMUNOLOGICAL PROBLEMS IN THE VIRUS FIELD.* Thomas M. Rivers, New York City.

Abstract. The most interesting pathological problems in the virus field have to do with the relation of the viruses to host cells. It appears that these active agents are obligate parasites and induce certain changes in the parasitized cells. Inclusion bodies often result from such an infection and a study of some of these structures has yielded valuable information. For instance, it has been clearly shown that certain inclusion bodies represent aggregates of minute structures which either are the virus or are closely associated with the virus. Hyperplasia,

* Presented at special request of the Council.

hyperplasia followed by necrosis, and necrosis are the most important phenomena in the pathological pictures produced by viruses. Inflammation is of secondary significance. In view of this fact one can easily understand why viruses enter into any discussion concerning the etiology of tumors.

Viruses are antigenic, and as antigens and because of the fact that an infection with them is usually followed by a lasting immunity they naturally have attracted the attention of workers in the field of immunology. One would like to know why it is so difficult to obtain a solid immunity with completely inactivated viruses. Also the differences in the duration of immunity produced by active and inactive viruses give rise to certain questions. Active viruses usually lead to a more or less prolonged resistance in a recovered host, while the immunity—often incomplete—induced by an inactive virus may be fleeting or at least of a relatively short duration. Moreover, in a few instances infections with viruses do not produce a lasting immunity.

Is it likely that there are special principles of immunity applicable only to virus diseases? There is no evidence that such is the case. The questions raised above might apply equally well to immunological phenomena in general, whether excited by bacteria, spirochetes, protozoa, or proteins of different sorts. The amounts of antigen, the multiplicity of antigens in an infectious agent the liabilities of which may vary, the intimacy of contact of the host with the infectious agent, and the duration of this contact all play a part in the immunological phenomena produced by all infectious agents. Certainly some of the immunological phenomena observed in virus diseases may, therefore, be explained upon the intimate type of parasitism exhibited by the viruses, the liability of certain antigenic components of these agents, and finally, the prolonged sojourn of the viruses in a host once infected.

EXPERIMENTS ON THE EPIDEMIOLOGY OF PSEUDORABIES. Richard E. Shope (by invitation), Princeton, N. J.

Abstract. Pseudorabies is a highly fatal but non-contagious disease in cattle and the common laboratory animals. It is a relatively mild but highly contagious disease in swine. It has been shown that in swine the nose serves both as the portal for the entrance and the exit of the virus. Furthermore, it has been found that fatal pseudorabies infections in rabbits can be induced by merely bringing their abraded skin into contact with the noses of infected swine. The blood serums of swine on two farms where pseudorabies had occurred among the cattle were studied and found to be capable of neutralizing pseudorabies virus. It is believed that in these instances the swine had suffered a mild and unrecognized pseudorabies infection and had probably transmitted their disease to the cattle with which they were associated.

To obtain information as to the incidence of pseudorabies among Middle Western swine, 23 samples of anti-hog cholera serum, representing blood serum from over 2500 swine, were studied. Twenty-one of these samples were found to contain pseudorabies virucidal antibodies. In 9 of the samples the antibody titer was such as to indicate that at least 5 per cent of the animals furnishing the serums had suffered an earlier infection with pseudorabies virus. The virucidal titer of the remaining 12 samples was such as to suggest upwards to a 50 per cent previous pseudorabies infection among the swine supplying the serums. The serums of 23 out of 25 adult swine of Middle Western origin contained pseudorabies virucidal antibodies. Serums obtained from local swine have been found

to be free of virus-neutralizing antibodies. It is concluded from the serological data that pseudorabies may be a highly prevalent, even though unrecognized, disease among Middle Western hogs. The experiments presented suggest that swine may serve as the source of infection for cattle, virus transmitting from the noses of infected hogs to the abraded skin of cattle.

VARIATIONS IN NEUROINVASIVENESS OF CERTAIN VIRUSES IN RELATION TO THE AGE OF SUSCEPTIBLE HOSTS. Peter K. Olitsky and (by invitation) Albert B. Sabin and Herald R. Cox, New York City.

Abstract. This study indicates that as an animal matures it may acquire resistance to certain neurotropic viruses, which depends neither upon previous exposure to infection and subsequent development of humoral antibodies, nor upon any change which age induces in the body as a whole, but rather upon a modification in certain special parts of the nervous system. The observations were made with the Indiana and New Jersey strains of vesicular stomatitis virus which are highly neurotropic in mice. The results in brief are as follows: (1) while young mice, 15 to 20 days old, readily develop a fatal encephalitis when the virus is dropped into the nose, old mice (about 1 year old), with only rare exceptions, show no signs of disease when given as much as 1000 to 10,000 times the minimal amount of virus which is fatal for the young ones; (2) yet young and old mice are equally susceptible when the virus is injected directly into the brain; (3) the resistance of the old mice is not dependent upon the presence of antiviral bodies in the blood; (4) the resistance becomes appreciable between the 20th and 30th days of life; (5) mature mice reveal a similar resistance when the virus is given subcutaneously instead of intranasally; (6) mature mice exhibiting no signs of disease following intranasal administration of virus, nevertheless develop specific humoral antibodies and a specific active resistance to intracerebral inoculation of the virus.

The development of the specific immunity in the mice which showed no signs of disease suggested that an unrecognized infection had occurred somewhere in the body. No virus was found in the blood, lungs, liver and spleen, thus excluding these as foci of infection. Since a direct inoculation in the brain is equally fatal to young and old, it appeared that the difference observed between the different age groups when the virus is introduced by way of the nose might be due to a resistance encountered by the virus somewhere along its course between the nasal mucosa and the important centers in the brain. It remained to determine whether the barrier was pre-ganglionic, *i.e.* in the olfactory nerve fibers with a resultant inability of the virus to reach the olfactory bulb or ganglion; or post-ganglionic, when the virus might reach the olfactory bulb but be unable to spread to the rest of the brain. Experiments revealed that apparently the resistance is chiefly post-ganglionic in the sense just indicated. Whereas in young mice the virus from the nose extended first to the olfactory bulb and then rapidly to the rest of the brain, in mature mice it also invaded the olfactory lobe but failed to spread to the rest of the brain.

It should be pointed out that with viruses which have a high initial invasiveness for the host, like Eastern equine encephalomyelitis in mice, and pseudorabies in guinea pigs, no appreciable difference was seen in their behavior in animals of different ages. It is not improbable that the well known resistance of older age groups in the case of poliomyelitis in man may at least in part be due to the phenomenon just described.

Discussion

(Dr. William Boyd, Winnipeg.) Dr. Rivers, in the remarkable review he gave this morning, pointed out that not very much attention had been paid to the histological or cytological changes in our morning symposium. I should like to ask Dr. Sabin if any histological investigations were made. Does one find in the non-immune, that is to say, in young animals, definite changes, either inflammatory or in the nature of inclusion bodies, and are these absent in the adult?

(Dr. Stuart Mudd, Philadelphia.) One of the slides seemed to imply that you could get passive protection in young mice with the serum of adult mice. Is that true?

(Dr. Sabin, closing.) Histological examinations were made, but unfortunately this is a virus disease which does not give rise to very characteristic cytological changes. No inflammatory changes, other than some dubious increase in cells in the olfactory portion of the brain, were found. The brain and nasal mucosa of the adult resistant mice were sectioned, but they showed nothing which would aid in determining the focus of virus action.

In reply to Dr. Mudd, the slides showed that there is a passive protection in that adult mice which had exhibited no signs of the disease developed antibodies in their serum which protected young mice against 1000 infective doses by the cerebral route.

(Dr. Mudd.) Does the serum of normal adult mice confer protection on young mice?

(Dr. Sabin.) Normal adult mouse serums had no antibodies for the virus.

STUDIES ON INCLUSION BODIES OF A NEUROTROPIC VIRUS IN VARIOUS ORGANS.
Abner Wolf and (by invitation) Margaret Holden, New York City.

Abstract. The W virus was originally isolated by Drs. Gay and Holden from a case of fatal ascending myelitis in a laboratory worker bitten by a monkey. A series of rabbits were injected with this virus in the parotid and submaxillary glands, adrenals, liver, spleen, kidney, testis and skin. In each of these organs lesions were produced which were characterized by necrosis, inflammation with occasional hemorrhage, and in all but the liver and spleen intranuclear inclusions. Hyperplasia was noted in the skin and connective tissue. In each case the infection spread to the nervous system. It involved the spinal cord first when the abdominal viscera or abdominal skin was injected, as evidenced by the limb paralysis; or the brain directly when the parotid or submaxillary were injected, as shown by convulsions. In those instances in which the abdominal organs were injected there was a peritonitis, intense in most cases and slight in only one. Blood vessels in the necrotic organs, in particular the adrenals, showed an intense arteritis with intranuclear inclusions. Connective tissue, subcutaneous fat and striated muscle about the salivary glands were affected. There were intense inflammation and necrosis in the former two and intranuclear inclusions and moderate degeneration were observed in the latter. The nerves in the organs injected always showed inflammatory changes in the perineurium and often endoneurium; occasionally they exhibited necrosis, and at times intranuclear inclusions were found in the Schwann and endoneurial cells.

Intranuclear inclusions were most frequent in the adrenals and salivary glands; fairly common in the central nervous system and rare in the kidney, testicle and skin. They were also encountered in striated muscle, blood vessel

walls and the Schwann and endoneurial cells of nerves. They were not found in the liver and spleen in our animals although seen in 1 case in the connective tissue cells of the markedly infiltrated capsule of the latter.

They varied considerably in type and intensity of staining reaction. In the adrenal they were homogeneous, deeply eosinophilic, oval or spherical, and tended to fill the entire nucleus. The chromatin was margined in every case. Occasionally a partial halo could be seen about the inclusion bodies. In the salivary glands they varied from multiple small inclusions to single large bodies which always had a halo about them. They were rather lightly eosinophilic and somewhat irregular, rather than oval or spherical. In general they were coarsely granular rather than homogeneous. In the kidney and seminal vesicle the inclusions resembled those seen in the salivary glands. In the skin some of the epidermal cells contained small, irregular, deeply eosinophilic intranuclear inclusions which were much like those seen in glial cells to be described later. They were either large, single and homogeneous, or multiple and separated often by bars of chromatin. As found in striated muscle, the inclusions were single, small, very deeply eosinophilic, homogeneous, oval or spherical, and had halos about them. In Schwann and endoneurial cells they resembled closely the inclusions seen in the adrenals, while in blood vessels they were more like those seen in muscle.

In the nerve and glial cells, the spinal cord and brain, inclusions of all the varying types described above were seen. In general the inclusion bodies in the nuclei of the nerve cells were homogeneous, deeply eosinophilic and had halos about them. Very frequently they filled the entire nucleus, as in the adrenal. Occasionally they were granular and irregular, the granules being rather uniform in size. Often they were small, compact, deeply staining and multiple. The cytoplasm of the affected nerve cells was always very pale staining and lacked density. In one instance this change was found in the cytoplasm of nerve cells in the basal ganglia without intranuclear inclusions. The animal had had cerebral symptoms, but showed neither infiltration nor inclusion bodies.

In the glia cells the inclusions were homogeneous and filled the nucleus or had a slight halo. Frequently, however, they were small, irregular, multiple and separated by bars of chromatin, as in the skin. In one rabbit injected in a number of abdominal viscera, there was the usual inception of nervous symptoms by a limb paralysis. There were no inflammatory changes in the spinal pia arachnoid or spinal cord. Numerous intranuclear inclusions associated with degeneration were observed in the glial and nerve cells of the cord, however.

The so-called B virus, isolated from the same human material by Dr. A. B. Sabin, is probably identical with the one here described.

Discussion

(Dr. Albert B. Sabin, New York City.) This virus, the pathology of which Dr. Wolf has just described, is a very interesting one. It was isolated simultaneously from the same case by Drs. Gay and Holden, and by myself. They called it W virus; I called it B virus. I see by this work that the two viruses which we have studied individually are apparently identical, but at one time they expressed the opinion that their W virus was herpes. I should like to know whether further studies since then have caused them to change their minds, and whether the description just given is for herpes or for a distinct virus. I have since then published the results of an extensive biological and immunological investigation

which showed that while the B virus is related to herpes and pseudorabies, it can be distinguished clearly from them by serological and biological methods. One of the important biological distinctions from herpes is that the B virus is pathogenic for the *Macacus rhesus* monkey. It may be recalled that the human disease followed a bite by a rhesus monkey. It has been possible to show that apparently the B virus causes a mild natural disease in the rhesus monkeys, while when transmitted to more susceptible hosts it produces a disease and lesions of the same type seen in man and the rabbit. It belongs to a group of viruses which might be termed pantropic, because of their pluricellular affinities.

(Dr. William Boyd, Winnipeg.) Are the lesions in all the organs purely degenerative, or did those in the brain show any inflammatory changes in addition to the degeneration?

(Dr. Wolf, closing.) In answer to Dr. Sabin, to my knowledge there has been no further work by Gay and Holden on the question of the relation of this virus to the herpes virus. My own interest in the problem is in the pathology and from that point of view I consider that this W virus is identical with the B virus.

In the majority of the organs inflammatory changes were present. But these were not marked and did not form an important part of the picture. In one rabbit intranuclear inclusions were found throughout the cells of the spinal cord, but no inflammatory reaction at all.

SHWARTZMAN PHENOMENON IN VACCINE VIRUS LESIONS. Lewis Henry Koplik (by invitation), New York City.

Abstract. Recently Gratia and Linz * elicited the Schwartzman phenomenon by combined intradermal injections of testicular vaccine virus and intravenous injection of *B. coli* culture filtrate. It was considered of interest to determine whether vaccine virus cultured *in vitro* would have a skin-preparatory potency and, furthermore, whether the intravenous injection of vaccine virus preparations following an intradermal injection of the culture virus would elicit a local response at the site of vaccination.

Vaccine virus was cultured according to the method of Rivers (Rivers, T. M., *J. Exper. Med.*, 1931, **54**, 453-461, and Li, C. P., and Rivers, T. M., *J. Exper. Med.*, 1930, **52**, 465-470). *B. typhosus* culture filtrate was made as described by Schwartzman (Schwartzman, G., *Proc. Soc. Exper. Biol. & Med.*, 1929, **26**, 843-845). Stock rabbits were used. They were each injected with 0.25 cc. of cultures of vaccine virus fresh or glycerinated. From 1 to 8 days later they received an intravenous injection of *B. typhosus* culture filtrate, of vaccine virus culture or of neurovirus. The effect of repeated intradermal injections of vaccine virus cultures was also noted.

Following the intravenous administration of *B. typhosus* culture filtrate (20-40 units per kilo), of culture virus (1-2 cc. per kilo), or of neurovirus (0.1 cc. per kilo) to rabbits vaccinated with culture virus 3 to 6 days previously, there was a marked change in the appearance of the local lesions in such rabbits as compared with control rabbits. The vaccinia vesicles and pustules became surrounded in 5 to 20 hours by areas of hemorrhage into the skin and subcutaneous tissue. These changes were observed in 60 per cent of the animals so treated but not in the controls. This phenomenon was not observed when the intrave-

* Gratia, A., and Linz, R. *Compt. rend. Soc. de Biol.*, 1932, **108**, 238.

nous injection followed the intradermal after 1 or 2 days or in the healing state (*i.e.* after 7 or 8 days). A second intradermal injection of culture virus after an interval of 3 days at the site of the first did not produce visible hemorrhage around the vaccinia lesion. Culture virus heated to 58° C. for 10 minutes was also ineffective in preparing the skin or in producing a state of local reactivity when given intravenously. Glycerine alone did not act as a skin-preparatory factor, and testicular extract caused only a localization of the vaccine virus, if intradermal inoculation of such extract was followed within 6 hours by an intravenous injection of the virus.

From our results it is evident that vaccine virus cultures are effective in preparing the skin of rabbits for the Schwartzman phenomenon when the intradermal inoculation of such cultures is followed after an interval of 3 to 6 days by an intravenous injection of similar culture virus, of neurovirus or of *B. typhosus* culture filtrate. The virus must be potent and an active lesion is essential for the elicitation of hemorrhage at the local site.

PATHOLOGICAL ASPECTS OF THE LOCAL AND GENERAL SCHWARTZMAN PHENOMENON. I. E. Gerber (by invitation), New York City.

Abstract. Detailed histological studies of the local Schwartzman phenomenon were previously made by Karsner and Moritz, Apitz, and Kielanowski and Selzer. The present study was undertaken in order to determine the sequence of events in the appearance of the hemorrhagic reaction in the skin. Experiments were performed with bacterial filtrates of ascertained skin-preparatory potency in various dilutions. The skin preparation was followed by intravenous injections after various intervals of time. The actions were studied at various periods. It was observed that no parallelism existed between the degree of inflammation following skin preparation alone and that following the appearance of the phenomenon. A slight degree of preparatory inflammatory reaction may be followed by a marked hemorrhagic and inflammatory response upon intravenous injection of the bacterial filtrate. The reaction in the skin after the intravenous injection is out of proportion to the inflammation that results from mere augmentation of the preparatory inflammatory reaction by repeated skin injection alone.

Apitz recently described the general Schwartzman phenomenon obtained by means of two successive intravenous injections of bacterial filtrate 24 hours apart. Each intravenous dose varied from 0.5 to 5.25 cc., or were given so that a total of 0.7 cc. was administered in 3 divided doses on the 1st day and a total of 6.3 cc. in 3 divided doses on the 2nd day. The internal organs showed diffuse vascular and concomitant degenerative changes. In the present study a series of rabbits received two successive intravenous injections of bacterial filtrate, with an interval of 24 hours between the injections. The potency of these filtrates was ascertained by their ability to elicit the local Schwartzman phenomenon. The doses ranged from 50 to 500 reacting units, *i.e.* 0.005 to 1 cc. Some rabbits also received skin-preparatory injections simultaneously with the first intravenous injection. Widespread pathological alterations were observed in the internal organs. These consisted of diffuse vascular changes in the kidneys, lungs, and liver, and apparently concurrent degenerative changes in the heart, liver and kidneys.

Discussion

(Dr. Eugene L. Opie, New York City.) It seems to me doubtful if these experiments exclude the possibility that inflammation has a significant part in the production of the Shwartzman phenomenon. The tests that are described show that substances causing the Shwartzman phenomenon are inflammatory irritants. The experiments of Menkin and a number of others have shown that some readily recognizable substance, such as trypan blue injected into the circulation, localizes in an inflamed area when the dye is used to produce a conspicuous blue spot. If a similar process occurs with the reactions that have been described, the vaccine of the second injection may accumulate at the site of the first injection and reaching it by way of the vascular system may produce the hemorrhage and thrombosis that accompany the Shwartzman phenomenon.

(Dr. Howard T. Karsner, Cleveland.) I am very much gratified that Dr. Opie made the statement that he did, and I hope that what he had to say will allow Dr. Gerber to continue what he undoubtedly thought of in connection with the general Shwartzman phenomenon. However, I do not think that what Dr. Gerber said alters the statement which has been made by several people, including Moritz and myself, that the inflammation which succeeds upon the injection of the protective factor is different from that which follows the primary injection, only quantitatively and not qualitatively.

(Dr. Gregory Shwartzman, New York City.) I should like to draw Dr. Opie's attention to the fact that a number of various substances of non-bacterial origin were tested by me and other investigators. Not a single one was shown thus far to produce the necessary state of reactivity in the rabbit. Dr. Freund reported in a recent publication that silver nitrate, which produces primary hemorrhages, also elicits the state of reactivity in the guinea pig. The author did not state whether he tested this preparation in rabbits. So far, I have tested 45 rabbits with various dilutions of silver nitrate. Silver nitrate usually produces a primary necrotic lesion, but no effect is produced by the subsequent intravenous injection of a potent bacterial filtrate upon the prepared site.

The various non-bacterial substances which were used for skin preparation are those capable of eliciting the severest type of primary inflammation (turpentine, arsenic, and so on); those producing acute vasodilatation, vasoconstriction, and vasoparalysis (histamine, adrenalin, acetylcholine, calcium chloride, urethane ethyl, and so on); antigen-antibody complexes (horse serum plus anti-horse rabbit serum, eggwhite plus eggwhite antiserum, and so on), various substances blocking the reticulo-endothelium system and the like. None of approximately 60 of the various non-bacterial substances employed ever produced any state of reactivity. In recent work, Kielanowski excised small portions of skin at various periods of time after preparatory injection. Rabbits were then given the provocative injection. The remaining non-excised portions of the prepared site served as a control as to the susceptibility of the animal to the phenomenon. In his extensive histological studies he found that the degree of primary inflammation bore no relationship to the reaction produced by the subsequent intravenous injection. Moreover, Apitz studied the inflammation produced by bacterial filtrates in animals refractory to the phenomenon (rats). It is extremely interesting that the degree of primary inflammation observed was the same as in susceptible animals. In addition, there are a number of various bacterial filtrates which produce a considerable degree of primary inflammation

(staphylococcus, streptococcus, pneumococcus filtrates, and so on) and yet completely fail to elicit the state of reactivity.

It is perfectly true that fixation of particulate matter by inflamed tissues clearly postulated by Dr. Opie may play an important rôle in the localization of the toxin from the blood stream. Naturally, the reacting factors must localize in the tissue in order to produce an effect, but there is no doubt whatsoever that there must be recognized also a peculiar type of a reactivity in the tissues elicited by special bacterial factors. This reactivity is quite apart from the incidental inflammation. Furthermore, there must be recognized also some additional dynamic factors which would be responsible for the production of the dramatic lesion following their localization.

(Dr. Theodore J. Curphey, New York City.) I should like to ask Dr. Gerber whether he made any platelet counts on the rabbits at the height of the reaction in an attempt to explain the thrombi that they show.

(Dr. Gerber, closing.) In answer to Dr. Curphey, we have not done any hematological studies on these animals.

TRANSMISSION EXPERIMENTS OF THE VIRUS OF POLIOMYELITIS IN MICE. Maurice Brodie, Samuel Goldberg and (by invitation) Phyllis Stanley, New York City.

Abstract. The mouse was used in this work owing to the fact that the virus of poliomyelitis survives for a longer time in the mouse brain than in that of the other small laboratory animal.

Three series of mice were subjected to 7 or more short doses of X-ray, following which they were injected intracerebrally and intraperitoneally with poliomyelitis virus. In the first series on the 11th and 12th day after injection the mice were slow, weak and had ruffled hair. Inoculation into mice and a monkey of a suspension of the brains of several of these mice produced no reaction. The second series of mice showed symptoms similar to those of the preceding series after the same interval of time following injection of the virus. Untreated mice injected with a suspension of these brains showed identical symptoms but with a shorter incubation period. The virus has been carried through 17 generations in mice, during which time the incubation period has become shortened to 3-4 days, the infectivity of the virus for the mouse increased and the symptoms have become more clear-cut. The injection of the suspension of brain material from the original mice of this series, into a monkey, produced a rise in temperature, but in the next passage in mice gave what seemed like typical poliomyelitis. Passage 3 also gave the typical disease in monkeys, while with passage 7 and 8 specific neutralization was obtained with three specimens of serums having poliomyelitis antibodies.

The mice of the third series behaved like those of the second. Passage of their brain material into untreated mice produced similar symptoms. A suspension of their brains produced a typical poliomyelitis when injected into a monkey and on the 2nd passage up to a 1:5000 dilution and on the 8th passage to a 1:1000 dilution of the mouse brain. During the transmission of the virus of this series through 14 generations of mice, it has undergone changes in incubation period, infectivity and symptoms produced, identical with those of the preceding series. Specific neutralization was obtained up to the 16th passage.

In the mouse, the disease produced by the injection of this virus is acute. It is evidenced by irritability, ruffled hair, ataxia, humped back, convulsions, twisting

of head and death. In the mouse, the pathology is found mainly in the brain and meninges. In the subarachnoid space it consists of mononuclear infiltration which is mainly perivascular. Perivascular collars, areas of hemorrhage, focal areas of necrosis and glia reactions are seen in the cerebrum. The spinal cord and brain stem show an occasional perivascular collar and some hemorrhagic foci. The cerebellum appears normal. The distribution of the virus shows some correlation with the histopathological picture. We appear to have transmitted the virus of poliomyelitis for the following reasons:

1. The virus has been successfully transmitted from mouse to monkey and from monkey to monkey and mouse.
2. Serum from convalescent and actively immunized monkeys and humans neutralized the virus.
3. The neutralizing power of various serums appeared to correlate when tested both in mice and in monkeys.

In the 17th passage and thereafter of both series the mice came down in the usual incubation period and with what appeared to be the same symptomatology. However, the disease incitant was no longer neutralized by poliomyelitis neutralizing substance, nor did it produce paralysis in monkeys.

Discussion

(Dr. J. Furth, New York City.) Is the application of repeated small doses of X-rays preferable to a single massive dose? It has been found in our laboratories that a single massive dose of hard X-rays increases susceptibility to several agents and inflicts profound damage upon the blood-forming organs. To understand the mechanism of increased susceptibility to the virus of poliomyelitis, it would be significant to know whether soft X-rays are necessary to produce it.

(Dr. Albert B. Sabin, New York City.) I should like to ask two questions: first, is the mouse passage virus pathogenic for guinea pigs and rabbits, and second, is a monkey convalescent from monkey passage virus resistant to mouse passage virus, and is a monkey convalescent from mouse passage virus resistant to monkey passage virus?

(Dr. Edwin W. Schultz, Leland Stanford University, Calif.) I should like to ask whether the anterior horn cells showed neuronophagia.

(Dr. Brodie, closing.) We do not know whether a single large dose of X-ray would be any better than repeated doses, because we did not try it. The only thing we tried to do was to get a block of the reticulo-endothelial system by a single large dose of India ink.

We have not been able to infect guinea pigs or rabbits, which I did not mention, with the virus.

In reply to Dr. Sabin's question, a monkey that recovered from mouse passage virus showed neutralizing substance for the monkey passage virus; we did not inoculate this animal directly intracerebrally, inasmuch as it died soon after being bled. Monkeys immunized with mouse passage virus showed specific antibodies which neutralized the monkey passage virus.

In reply to Dr. Schultz, even though the mice occasionally showed what appeared to be paralysis, they never showed sufficient in the spinal cord to account for it. We found no neuronophagia in the spinal cord. The only lesions we have found are mononuclear infiltration of the subarachnoid space and some perivascular infiltration in the gray and white matter, and some hemorrhage, but no nerve cell destruction. The spontaneous mice virus which we picked up looked

more like polio than the other because we found what appeared like definite destruction in the spinal cord.

TRANSMISSION OF EQUINE ENCEPHALOMYELITIS BY MOSQUITOES. Carl Ten Broeck and (by invitation) Malcolm H. Merrill, Princeton, N. J.

Abstract. Since in the East the great majority of cases of equine encephalomyelitis are found along the shore line, the ability of a number of salt marsh mosquitoes to transmit the disease has been tested. *Aedes sollicitans*, the salt marsh mosquito found most abundantly in the regions where the disease occurs, can be infected by feeding on infected brain mixed with blood, or on an infected guinea pig or horse. It apparently retains the virus as long as it lives and regularly transmits it by biting. Transmission of the virus from infected to normal guinea pigs and from infected horses to normal guinea pigs and a horse has been obtained.

Further experiments, not so extensive, indicate that *Aedes cantator*, *Aedes taeniorhynchus* and *Aedes vexans* will also act as transmitting agents. The last is a fresh water mosquito which may be involved in the transmission of the Western type of the disease. Transmission experiments using *Culex pipiens* and *Anopheles quadrimaculatus* have been uniformly negative.

In making transmission experiments it is important that mosquitoes be fed on material containing virus of high titer. When the titer is low the virus can be demonstrated immediately after feeding by inoculation of a suspension of crushed mosquitoes, but inoculation and feeding experiments at 5 days and thereafter are negative.

Discussion

(Dr. Marshall Hertig, Boston.) I should like to inquire the method by which the mosquitoes were fed the suspension of brain and blood.

(Dr. Ten Broeck.) The mosquitoes were starved for 4 days and kept for 24 hours without water, after which the virus was offered them and they took it readily.

(Dr. Hertig.) How was it applied?

(Dr. Ten Broeck.) On a piece of cotton.

FURTHER STUDIES ON THE INFECTIVITY OF TRACHOMA. L. A. Julianelle and (by invitation) R. W. Harrison, St. Louis, Mo.

Abstract. Studies conducted in this laboratory reveal that trachoma is an infectious disease transmissible to monkeys. The specificity, course and nature of the experimental infection, together with the natural resistance of certain animals to trachoma, their lack of acquired immunity to the disease following infection, the inability to establish the infectious agent permanently in monkeys, and so on, have already been reported. Since that time the investigation has been devoted almost exclusively to the determination or demonstration of the causative agent of trachoma. The general method employed has been that of elimination and up to the present the following is the information gained from this study.

It was established very early that faulty or deficient diet plays no accessory part in the causation or evolution of the disease. Elaborate studies of the bacteria cultivable from trachomatous tissues indicate that the infectious agent is not bacterial, whether considered as a single specific organism or as several or-

ganisms associated in a non-specific infection. Further experiments revealed that under the usual conditions of filtration the infectious agent does not traverse Berkefeld filters.

It appeared, then, that the incitant of trachoma resides in that field flanked on one side by bacteria and on the other by filterable viruses. This includes, therefore, (1) the basophilic, heterogeneous, epithelial cell inclusion described by Prowazek and Halberstader, (2) Rickettsiae, (3) non-filterable viruses, or (4) some unknown and unsuspected agent. Epithelial cell inclusions are difficult of reconciliation because materials not containing inclusions are frequently infectious, and materials containing inclusions are frequently not infectious. Furthermore, despite numerous examinations, inclusions have never been found in preparations from monkeys infected experimentally.

Repeated examinations in the human and monkey have revealed no Rickettsiae, and cultivation by tissue culture methods has yielded thus far cultures completely devoid of bacteria or Rickettsiae, and even infectivity.

In attempting to acquire information on the non-filterable virus concept of the disease, it has been found that active trachomatous tissues preserve their infectivity for 2 weeks or more following inoculation in the rabbit and guinea pig testicle. The ground testicle is infectious for monkeys, inducing the typical signs of the experimental disease. The testicular material is bacteria-free; it shows no distinct pathological changes, grossly or microscopically; it exhibits no organisms or inclusions, and on tissue culture yields no growth of infecting organisms as determined by direct examination or inoculation of monkeys. Similar manipulation of normal testicle, or testicle inoculated with material from folliculosis of humans, from chronic conjunctivitis of unknown origin, and even from non-infectious trachoma induce no changes in the conjunctiva of monkeys. Simultaneous inoculation of active or inactive trachomatous tissues and normal testicle does not affect the original degree of infectivity of the material. It is not possible to interpret the significance of these experiments at the present time. The work is being reported not to define the infectious agent of trachoma, but merely to indicate the adoption of a new method in the study of the etiology of this disease. Whether this method will prove fruitful or not must wait upon further work.

THE PATHOGENICITY OF *BRUCELLA ABORTUS* FOR WHITE MICE. William H. Feldman and (by invitation) Carl Olson, Jr., Rochester, Minn.

Abstract. For the purpose of determining the pathogenicity of the cattle and swine varieties of *Brucella abortus* for white mice, 3 different strains of *Br. abortus bovis* and 3 of *Br. abortus suis* were used to inject a total of 36 animals. The mice were divided into three groups for the purpose of autopsy and one group was killed after 30 days, another after 44 days, and the third group after 70 days. Practically all of the animals survived the respective periods of the experiment and *Br. abortus* was recovered from the spleen of 28 or approximately 83 per cent of the 34 animals in which recovery of the organism was attempted. *Brucella* agglutinins of significant titer occurred in nearly all the animals whose blood was tested. The pathological anatomy was also studied and while gross manifestations of the disease were infrequently seen in the respective mice, characteristic microscopic lesions were present in most of the animals. The lesions which were essentially diffuse or focal accumulations of histiocytic cells of peri-

vascular inception occurred most often in the kidneys and liver. The spleen, testes and epididymis were affected less frequently.

Conclusions

1. Cattle and swine strains of *Br. abortus* when injected intraperitoneally are pathogenic for white mice.
2. Brucella agglutinins are present in the blood of the inoculated animals and the specific organism is recoverable from the spleen.
3. Although grossly visible evidence of a diseased state infrequently occurs, rather characteristic lesions of the kidneys, liver, and less frequently of the spleen, testes and epididymis, may be observed microscopically.
4. White mice should be satisfactory animals for the isolation of *Br. abortus* from spontaneously infected material.

THE RELATION OF ALLERGY, RESISTANCE AND ANTIBODIES IN ANIMALS VACCINATED WITH THE CALMETTE-GUERIN BACILLUS (B. C. G.). B. J. Clawson, Minneapolis, Minn.

Abstract. Allergy and resistance were studied in relation to each other and in relation to antibodies in the serums of animals vaccinated with B. C. G.

Allergy as discussed refers to the phenomenon illustrated by the skin tuberculin reaction.

Resistance was indicated by a partial or complete retardation of the progress of infection due to virulent strains of tubercle bacilli.

The antibodies studied were agglutinins, complement fixation antibodies, opsonins and lysins.

Methods and Materials: (A) *Allergy.* Rabbits were vaccinated so as to produce allergy in some but not in others. Allergic rabbits were desensitized and the antibody content of the desensitized rabbits studied. A method of injecting animals to produce a degree of allergy and a minimum or no measurable antibody content in the serums was also employed.

(B) *Resistance.* Resistance due to vaccination was determined by comparing the degrees of tuberculosis in normal and vaccinated animals following an inoculation with a lethal dose of a virulent strain of the tubercle bacillus. The antibody content was studied in vaccinated resistant animals.

(C) *Relation of Allergy to Resistance.* The three methods used for this study were: (1) to vaccinate animals so as not to produce allergy; (2) to wait for the allergy to disappear before giving the virulent injection; and (3) to compare the duration of allergy and resistance.

Results of Experiments: There appeared to be no definite proportionate or necessary relation between the presence of allergy, as manifested by the intravenous tuberculin skin reaction and antibodies in the blood.

Definite resistance against virulent injections was developed by vaccinating rabbits and guinea pigs with B. C. G. The antibody titers were uniformly increased in the vaccinated resistant animals.

No proportionate or necessary relation appeared to exist between the phenomenon of allergy and the immune state (resistance).

The degree of the titers of the antibodies tended to correlate the degree of resistance.

Discussion

(Dr. Max B. Lurie, Philadelphia.) I am very much interested in hearing Dr. Clawson's statement of the lysin effects on tubercle bacilli *in vitro*. It has not been stated whether the effects are due to the cells or to the serum in the system. Certainly if you take highly immune serum, if you take the sera or plasma of extensively tuberculous rabbits highly immune to tuberculosis, and incubate that with tubercle bacilli, you find that they actively support the growth of the bacilli even to an extent greater than that of normal plasma. Is it due to the cells or to the plasma in the mixture?

(Dr. Joseph D. Aronson, Philadelphia.) I am in accord with Dr. Clawson in that there may be no relation between allergy and resistance. However, I cannot agree that there exists a relation between resistance and antibodies. A number of years ago I carried out a series of experiments on goats and sheep, which were bronchoscoped and small amounts of tubercle bacilli were then sprayed into the trachea at irregular intervals. For a period of several years serological reactions and the tuberculin reaction were carried out on these animals. No correlation could be established between the antibody content, the sensitivity to tuberculin, the longevity of the animals and the extent of tuberculosis. The animal that lived the longest and had marked fibrosis of the tuberculous lesions of the lung had the smallest amount of humoral antibodies. I have not succeeded in modifying the course of tuberculosis in guinea pigs treated with large amounts of immune serum obtained from goats or sheep hyperimmunized with the tubercle bacillus. Nor was the course of tuberculosis modified by the injection of such antisera previous to injection of guinea pigs with virulent tubercle bacilli.

(Dr. L. Dienes, Boston.) I made some observations which correspond to those of Dr. Aronson. By the treatment of tuberculous guinea pigs with virulent tubercle bacilli strong antituberculous serums were produced which gave complement fixation with tubercle bacilli in the dilution of 1:2000 or higher. As large doses as 15 to 20 cc. of these serums exerted no protective action in guinea pigs. I have seen also that very strong allergy produced with killed tubercle bacilli is not necessarily associated with increased resistance. The slower or faster progress of the disease and the resistance to new infection seem to depend on many specific and non-specific factors. Probably some of these factors are yet unknown; their way of cooperation is also unknown. At present it seems best to keep our judgment in suspense in the problem of tuberculosis immunity.

(Dr. E. T. Bell, Minneapolis.) Whatever theoretical considerations there may be against Dr. Clawson's work, we must not lose sight of this main fact—that the great majority of these animals were completely protected against massive inoculations of tubercle bacilli, the guinea pig against the human strain, the rabbit against the bovine strain. The controls were riddled with tuberculosis, but the vaccinated animals for the most part showed no lesions whatever.

(Dr. E. L. Opie, New York City.) It seems to me that our methods of measuring sensitization are unsatisfactory because we depend on a skin reaction in which a variety of factors is involved. We use tuberculin prepared by prolonged heating, and very different from the antigenic substances associated with the living tubercle bacillus. In the reactions used to measure antibodies several factors may be involved, and though two of these factors may remain constant, another factor may vary. Hence we should not expect an exact complete parallel in the occurrence of the reactions that are associated with sensitization and

resistance in tuberculosis. I should like to know what was the method of measuring lysis.

(Dr. E. M. Medlar, Mt. McGregor, New York.) I should like to ask Dr. Clawson how long he kept his animals. Tuberculosis is a disease of long standing, and you cannot draw definite conclusions from experiments in tuberculosis unless you keep your animals for some time. Recently I have had rather an interesting result in which I inoculated guinea pigs with living B. C. G., and kept them isolated where there was no chance of their becoming contaminated with any other tubercle bacillus. I forgot all about them. They had been isolated for 2½ years when I finally sacrificed them. One of the animals was riddled with tuberculosis. It appeared perfectly healthy at the time of killing.

(Dr. Stuart Mudd, Philadelphia.) Had these B. C. G. cultures been maintained according to Calmette or had they been altered?

(Dr. Medlar.) It was an original Calmette culture which we received in 1929. This culture has always been grown on a bile-glycerin-potato medium, as recommended by Calmette.

(Dr. Clawson, closing.) In reply to Dr. Lurie's question, I first will answer Dr. Opie as to how lysis was determined. It probably may not be correct to say that I had a humoral antibody. The lysis here took place within the phagocytic cells. The cells were obtained by injecting 100 cc. of paraffin oil into the peritoneal cavity of a normal rabbit. In 4 days the material was taken out, which gave a very heavy suspension of almost pure mononuclear leukocytes. Equal amounts of those cells, plus serum at a dilution of 1:75, plus a measured amount of B. C. G. were put on a mixing wheel and rotated for 1 hour in an oven at 37° C. Then a measured amount was put onto a measured surface on a slide and fixed with methyl alcohol and stained with the acid-fast stain. The determination of the lysis was made by determining the absence of the organisms by acid-fast or non-acid-fast stain. I realize this is a rather crude method, and do not depend on it very much unless there is a decided change. A 50 per cent reduction was considered certainly significant. The lysis does not take place in the serum. The serum without the cells did not reduce the number of B. C. G.

Dr. Aronson brought up a few points which I think I should make some statements about. The fact that antibodies are not detectable in serum does not prove the absence of the potentialities of antibodies. I find animals which will not show antibodies at the time of protection. If they are injected intravenously and then examined the next day for antibodies, the antibodies will come back up almost as high as they were normally. This I think cannot be due to a new development of antibodies.

In regard to Dr. Opie's statement as to the methods of measuring, I realize the methods of measurement in all the work on allergy and the concentration of antibodies are very crude. However, with the animals I used 1 mg. of Old Tuberculin; it is of such potency that when 0.05 of a mg. is injected intracutaneously into a positive individual a strong reaction is elicited.

In answer to Dr. Medlar's question, how long do I keep the animals, I presume he refers to the time I keep the animals after injection with B. C. G. The animals are kept from 3 weeks to as long as 5 months after being injected with B. C. G. and never did I find any progressive lesions. I did find microscopic lesions in the lungs in the animals which were injected intravenously with living B. C. G. The other animals were kept from 90 to 110 days, and those that survived were killed.

BACTERIAL LOCALIZATION AND GROWTH IN NORMAL AND IMMUNE TISSUES.

Paul R. Cannon, Chicago, Ill.

Abstract. The object of these experiments was to determine the comparative ability of certain organs of normal and of actively immunized animals to remove living bacteria from the blood stream and to ascertain the effects of immunization upon the growth proclivities in the different organs. The method was to excise the organs aseptically at varying periods of time after the intravenous injection of the bacteria, followed by incubation at 37° C. for 22 to 24 hours, in order to allow bacteria present in a tissue at the time of death of the animal to grow into colonies. The tissues were then fixed, sectioned and stained to demonstrate the bacterial colonies. Broth cultures of *Staphylococcus aureus*, *B. typhosus* and pneumococcus type I were used in order to avoid the introduction of masses of microorganisms into the blood stream, injected in equal amounts, simultaneously, into a normal and an immunized rabbit. The animals were sacrificed at intervals of from 20 minutes to 24 hours.

The results may be summarized as follows:

1. Bacterial colonies of *Staphylococcus aureus* and *B. typhosus* developed readily in the liver, spleen, lungs, kidneys and blood of both normal and immune animals. The colonies were most numerous in the liver and spleen, and in the earlier stages much more numerous in the immune animals, showing both the concentrating activity of these organs and the ability of the bacteria to withstand any bactericidal action of tissue fluids to the extent of preventing abundant growth. In fact, staphylococci were able to grow in the blood within the cardiac ventricle of immune animals injected intravenously at least 18 hours before the animal was sacrificed. There was very little tendency for colonies to appear in the kidneys or lungs, although they were present in animals killed comparatively soon after intravenous injection, but in negligible numbers, as compared with those observed in the liver and spleen.

The conclusions drawn are that bacteria tend to accumulate and concentrate in the immune liver and spleen in greater numbers than in the normal liver and spleen under comparable conditions. Furthermore, the bacteria can withstand the bactericidal activity of the blood and tissue fluids to a considerable degree, and after the death of the cells can grow into colonies. Colonies do not develop in many organs with a rich blood supply, such as the lungs, kidneys, pancreas or myocardium, thus showing the relative inertness of ordinary capillary endothelium as a phagocytic tissue. The results indicate the predominant influence of phagocytic cells in the removal of bacteria from the blood and suggest the inadequacy of the blood and tissue fluids as bactericidal agencies after the death of the cells themselves.

Discussion

(Dr. Max B. Lurie, Philadelphia.) I was very glad to hear the report of this paper because for several years I have studied the fate of tubercle bacilli in various organs after intravenous inoculation of rabbits. By culturing known quantities of the different organs on egg media, and then determining the number of colonies that developed from a given tissue, it was found that 24 hours after injection the localization was exactly as Dr. Cannon has reported. The quantity distributed in the different organs is as follows: The greatest amount per weight of organ is found in the spleen, next to that in the liver, next to that the bone

marrow, which Dr. Cannon has not studied, next to that in the lung, and least of all in the kidney.

(Dr. E. T. Bell, Minneapolis.) Dr. Cannon no doubt realizes that this localization of the bacteria is due to the distribution of the reticulo-endothelial system. The liver and the spleen contain the greater part of this. The reticulo-endothelial cells take up the bacteria, which are held there. The absence of bacteria in the kidneys is due to the fact that the endothelial lining of the glomerular capillaries is extremely thin; there is practically no cytoplasm in any of these cells in the normal kidney. There is nothing in the glomeruli that can act as a phagocyte. Whenever a bacterium is found in the glomerulus it is either mechanically stuck to the capillary wall or taken up into a polymorphonuclear in the blood. This is the distribution of India ink when it is injected in small quantities.

(Dr. Stuart Mudd, Philadelphia.) I should like to ask whether there is a difference between survival and later history of the bacteria in the normal and immune animals.

(Dr. Theodore J. Curphey, New York City.) Was there any tendency for localization of the pneumococci?

(Dr. Cannon, closing.) In reply to Dr. Curphey's question, I would say that the use of pneumococci in this work was not very successful as the organisms would not grow into colonies but grew diffusely. I saw no tendency, however, for localization in the lung as compared with other organs.

In regard to Dr. Mudd's question, these animals were all sacrificed within 24 hours. My chief object was to see how bacteria would grow in the excised organs of immune animals rather than to observe variations in bacterial localization, but inasmuch as this method demonstrated localizing potentialities of different organs, such as Dr. Lurie has just mentioned from his work, and as we have found also by determining the germ-content of tissues or organs of animals injected intravenously with living bacteria, I thought it was interesting to see the correlation from another angle of approach.

The lack of phagocytic power of glomerular endothelium, as mentioned by Dr. Bell, is of interest and is not generally realized. A paper appeared on this subject within recent years in which the method of staining bacterial colonies after death in excised tissue was used. The author observed that when animals were killed after 10 minutes and others after 4 hours, more colonies were found in the kidneys at the end of 10 minutes than at the end of 4 hours, and concluded that this demonstrated a greater phagocytic power in the kidney in the 10 minute animal than in the 4 hour animal. It seems to me, as Dr. Bell stated, that the explanation is quite obvious: the organisms are circulating and being removed from the blood as they go through the liver, spleen and bone marrow, and being washed out of the kidneys, until eventually practically none is left in the kidney from which a colony can develop. The point I have been interested in is that endothelium, not only in the kidney but elsewhere, as in the pancreas, thyroid, muscle, and so on, is relatively inert, so far as its ability to engulf bacterial particles is concerned.

ON THE MECHANISM OF IMMUNITY IN TUBERCULOSIS. THE FATE OF LIVING TUBERCLE BACILLI WITHIN A LOCALIZED AGAR FOCUS AND THEIR DISSEMINATION IN THE BODY OF NORMAL AND IMMUNIZED RABBITS. Max B. Lurie, Philadelphia, Pa.

Abstract. Sterile 6 per cent agar in saline at pH 7.4 is melted, and when cooled to 48° C. is thoroughly mixed with a suspension of virulent tubercle bacilli in India ink or trypan blue. A portion is injected subcutaneously into normal and B. C. G. vaccinated or actively tuberculous rabbits; the other portion is cultured on egg media. At varying intervals after inoculation, the number of living tubercle bacilli present in a given amount of the agar coagulum and its surrounding capsule, as well as that present in the draining lymph nodes and internal organs of the normal and immunized rabbits, is determined. The fate of the bacilli is then correlated with the histological changes in the corresponding tissues.

It was found that the agar coagulum is broken up into particles by an exudate of fluid, fibrin and cells, and that the bacilli grow freely in the agar totally away from the cells in the normal animal. In the immunized animal they either do not grow at all or are markedly inhibited in their multiplication in this location. Under the same conditions tubercle bacilli impregnated in agar and kept at body temperature die completely in 11 days. These facts point to an extracellular inhibitory factor in immunity to tuberculosis. Yet agar mixed with plasma of normal or tuberculous rabbits equally supports the growth of the contained bacilli *in vitro*.

In the normal animal the bacilli grow dispersed as well as in large, loose colonies. In the immunized animal they either persist in their original form without multiplication or grow as minute, dense clumps. This clumping is at least partly non-specific, for the same relations obtain with particulate matter such as carbon, which is largely agglutinated in dense masses in the tuberculous animal, whereas in the normal animal the carbon particles are largely dispersed and the agglutinated masses are less frequent and of looser texture. This is to be correlated with the greater retention of carbon at the site of reinfection than at the site of primary infection.

The bacilli in the normal animal penetrate the surrounding tissue and multiply unhindered within the cells. In the immunized animal there is little penetration of the capsule by the bacilli and those that do are actively destroyed by the cells.

However, all these factors which tend to prevent the spread of the bacilli from the site of reinfection are insufficient at first, and in the first days, due to the greater intensity of the inflammation and the increased lymph flow, more tubercle bacilli reach the immediate regional nodes in the reinfecting than in the normal animal. But, due to the greater capacity of the cells to destroy the microorganism, its growth is markedly retarded and they soon disappear in the immunized animal while they multiply unhindered in the normal animal.

The deeper lymph nodes and the internal organs of the sufficiently immunized animal practically completely destroy the few invading bacilli that reach them, whereas the large numbers invading those organs in the normal animal continue to multiply.

Discussion

(Dr. Paul R. Cannon, Chicago.) I should like to ask Dr. Lurie what is his impression of the cause of the greater extension of the tubercle bacilli to the regional lymph nodes in the tuberculous animals as compared to the normal.

(Dr. Calvin G. Page, Boston.) What kind of egg culture medium was used?

(Dr. Lurie, closing.) The explanation for the more rapid dissemination of the tubercle bacilli from the agar focus in the tuberculous animals is to be associated with the much greater degree of inflammation at the focus and the much greater flow of lymph from the site of reinfection in the tuberculous animal, as compared with that in the normal; this increased lymph flow tends to sweep away the bacilli with it. The fixation which is apparent later does not operate effectively in the early stages of the tuberculous reinfection.

In reply to Dr. Page's question, I have used Lowenstein's medium as a base, in which bone marrow infusion replaced the distilled water, as required by the original formula of Lowenstein.

CULTURAL AND PATHOGENIC PROPERTIES OF A NEW PATHOGEN ISOLATED FROM HUMAN CASES OF MENINGO-ENCEPHALITIS. Caspar G. Burn (by invitation), New Haven, Conn.

Abstract. An unidentified bacillus has been isolated during the past 18 months at the New Haven Hospital from four individuals, three infants and one adult. It was isolated both clinically and at postmortem in pure culture in the three infants. In the case of the adult it was found to be in association with a pneumococcus type III. The pathology consisted of multiple foci of necrosis and exudation in the liver and occasionally a similar lesion was found in the other organs. Two of the individuals showed central nervous system involvement consisting of hemorrhage and suppurative meningo-encephalitis.

The organism is a Gram-positive, non-spore-forming bacillus appearing singly, in clumps and occasionally in short chains. The colonies on blood agar plates resemble those of a hemolytic streptococcus, differing, however, in that the colonies are larger, flatter and more translucent. The usual sugars are fermented without gas, except for a delayed fermentation in lactose and glycerin.

The results with animal inoculations vary with the species of animal employed, the number of bacilli introduced and with the route of inoculation. Intravenous injection in rabbits varying from 100,000 to 10 million per cc. resulted in paralysis, convulsions and meningeal irritation on the 4th to 5th day and finally caused death. At autopsy, the liver showed diffuse spottings with focal zones of necrosis. In the central nervous system, the lesions revealed an extensive meningo-encephalitis in which subarachnoid hemorrhage occurred in 20 per cent of these rabbits. Monkeys developed similar liver and brain lesions, but larger quantities of bacilli were required. On the other hand, guinea pigs failed to develop central nervous system involvement upon intravenous inoculation, but instead consistently showed myocardial abscesses which resulted in death in 14 to 20 days.

Carriers were not found in two of the families in which epidemiological studies were made.

Further studies concurring the unusual affinity which these organisms have for the central nervous system are now in progress.

Discussion

(Dr. Edwin W. Schultz, Leland Stanford University, Calif.) I should like to add a few words to what Dr. Burn has said. About a year ago we reported in the *Proceedings of the Society of Experimental Biology and Medicine* similar observations on a case of meningo-encephalitis in a nurse at the Veterans' Hospital at Palo Alto. An interesting feature of this case was that the organism was recovered from the spinal fluid in pure culture 12 times over a period of more than 3 months. The cultural and experimental observations which we made seem exactly like those described by Dr. Burn. The nurse still has residual symptoms. We are carrying out further studies on this organism experimentally. I should like to ask Dr. Burn whether he has demonstrated motility in these organisms. We have not been able to demonstrate any motility.

(Dr. William Boyd, Winnipeg.) Are these sporadic cases?

(Dr. Burn.) Yes.

(Dr. Arthur W. Wright, Albany.) I should like to ask Dr. Burn if serological and immunological studies were made with the organism which he isolated; and also to ask Dr. Schultz if any studies have been carried out with the blood of the nurse who survived the infection, to determine whether or not her blood serum contained antibodies which were specific for the infecting agent.

(Dr. Schultz.) We checked all the cultures which were isolated from the nurse during this period culturally and serologically against sera produced in rabbits. I do not recall the extent to which the nurse's serum agglutinated, but it did in a low dilution eventually.

(Dr. Burn, closing.) In reply to Dr. Schultz' question about the motility, I have also been unable to demonstrate any evidence of motility; with various flagella stains used I have been unable to show any evidence of their presence.

With regard to the serological and immunological studies, we did some, and are carrying out more at the present time. In the rabbits surviving after a 5 day period, that is, those inoculated in small quantities, there will be evidence of agglutinins in the blood of these animals ranging anywhere from 1:20 to 1:100. Also monkeys, though they were more resistant, and larger quantities of bacilli had to be used to produce infection, showed agglutination in dilutions from 1:100 to 1:150 in their sera. We are also carrying out some serological tests with other strains of organisms we believe culturally related to this organism, and believe it agrees in every respect culturally and I think in some respects to the pathogenicity of the organisms described by Murray and Webb which they isolated from rabbits in 1925. Culturally we cannot differentiate them at all. Our strain also produced a definite monocytic response in rabbits within a period of 4 to 5 days, just at the height of the meningitis. Other studies are being made along these lines.

A NEW SPECIES OF THE GENUS *MONOSPORIUM* ASSOCIATED WITH CHRONIC OTOMYCOSIS. David L. Belding and (by invitation) Carl B. Umanzio, Boston, Mass.

Abstract. The association of a species of the genus *Monosporium* with a chronic infection of the ear is recorded for the first time. In the literature this genus has been reported chiefly in connection with Madura foot. The external auditory canal was lined with crusts, fine white scales, and moist macerated tissue. There

was a thin yellow to thick creamy discharge with an offensive odor. The morphology and cultural characteristics of the fungus indicate that it is a species distinct from those that have been reported in man.

THE BACTERIAL FLORA ASSOCIATED WITH FOREIGN BODIES IN THE TRACHEA AND BRONCHI. Carl Joseph Bucher, Philadelphia, Pa.

Abstract. Two hundred and forty-three specimens of mucopus obtained bronchoscopically from the tracheobronchial tree of patients who had a foreign body there were cultured. A study was made of the bacteria recovered in cultures and the type of foreign body, the age of the patient, and the sojourn in the lung, to determine how much importance was to be attached to them. It was concluded that the bacterial flora were less important in this respect than the nature of the foreign body, the degree of obstruction produced, location in the air passages, the sojourn there, and the age of the patient.

Discussion

(Dr. Calvin G. Page, Boston.) I have cultured bronchial mucus from more than 150 cases with the intention of growing fungi, if they were present, using three special sugar media. I should like to ask the speaker if he attempted to cultivate fungi. The cases I studied were mostly of routine bronchoscopy for tumor and so on, and not for the removal of foreign bodies. The idea was to try to find fungi, and I found them in only a few cases.

(Dr. Bucher, closing.) In addition to the organisms reported on the chart, occasionally there were Monilia. I suppose they may be classed as fungi. They were the only fungi found. I have cultured several thousand bronchoscopic specimens, and they occasionally occurred.

THE VISCERAL PATHOLOGY IN SCARLET FEVER. Henry Brody (by invitation) and Lawrence W. Smith, New York City.

Abstract. The paper presents a study of 61 autopsy cases of scarlatina and related streptococcic infections. It includes a histological report on the non-suppurative, toxic manifestations of the disease in the various viscera. The lesion is an interstitial one, consisting of an exudate of round cells, including chiefly lymphocytes but also many plasma cells, and other large monocytes. The lesion has been found in almost all of the body tissues. It is not, we believe, the result of direct injury to the interstitial tissue, but rather, primarily, a widespread injury to the vascular endothelium with, secondarily, fluid and cellular exudation.

Lesions of varying severity are found in over 90 per cent of the hearts, in these cases, principally as infiltration about the small coronary arteries or as a subendothelial infiltration of the coronary veins and the endocardium. Similarly, over 80 per cent of the kidneys show these lesions which are primarily of interstitial mononuclear infiltration in the boundary zone between the cortex and medulla. In the more extensive cases the picture resembles, both in gross and microscopically, lymphatic leukemia.

The liver is the next most frequently involved organ, the changes being noted in over 70 per cent of the cases. They are found principally around the smaller vessels in the portal areas, but extend out into the parenchyma to some degree.

Similar lesions about the veins and capillaries have been seen in the spleen, adrenals, pancreas, lung, pituitary, testis, tissues of the pharynx, regional and distant lymph nodes, salivary glands and aorta.

Discussion

(Dr. Virgil H. Moon, Philadelphia.) I have been much interested in the lesions in various organs resulting from scarlet fever infection, and am interested in Dr. Smith's interpretation of them as due to toxic effects rather than as due to the direct infection of the tissues by the organisms. The lesions in the liver which he states are particularly specific call to mind an instance in which in three children in the same family cirrhosis of the portal type developed following scarlet fever. In one of these cases there were such lesions as are shown here, but more marked, leading to typical portal cirrhosis with ascites. In this case streptococci were cultivated from the liver and were demonstrated in large numbers in the sections of the liver. I offer this suggestion, that perhaps in certain cases the lesions described may not be due to toxins elaborated by the organisms, but may be due to the presence of organisms in the tissues. I should like to ask Dr. Smith whether he made examinations to determine if bacteria were present in or about the lesions.

(Dr. Stuart Mudd, Philadelphia.) I should like to ask what the incidence of positive blood cultures was in this group.

(Dr. Otto Saphir, Chicago.) There are two questions I should like to ask. The first is, are the lesions which Dr. Smith showed so beautifully the direct result of whatever causes scarlet fever, or are they the result of the complications of scarlet fever? This might be answered by stating the time interval between the onset of the disease and death. The second question is, did Dr. Smith find any circumscribed proliferative lesions in the myocardium which resemble the so-called Aschoff bodies?

(Dr. E. T. Bell, Minneapolis.) I should like to ask Dr. Smith why he attributes this to endothelial injury. The lesions here are proliferative and exudative, especially in the walls of the arteries. They resemble the lesions which may be obtained experimentally by the injection of streptococci. I see no reason to consider this an endothelial reaction, such as we get in some of the virus diseases, like typhus and Rocky Mountain spotted fever.

(Dr. Howard T. Karsner, Cleveland.) I think that studies such as Dr. Smith has made are of unquestionable value in enlightening us as to the pathology of scarlatina. It occurs to me that the principal lesions he has shown are in relation to the blood vessels, and the study of the vascular system in various types of acute infectious diseases, which has gone on for many years, has shown that in many of these diseases lesions of this general character occur. In reference to arteriosclerosis, one wonders what bearing this sort of inflammation may have on subsequent disease of the vascular system. These lesions which Dr. Smith has shown are of great interest, but when Dr. Smith states that he sees something specific in their morphological character I fail to follow him, and I should like to have him explain further what he means by that. I see Dr. MacMahon sitting here, and I hesitate to speak of the resemblance found in the kidney sections to the picture seen in malignant nephrosclerosis.

(Dr. H. E. Robertson, Rochester, Minn.) I am interested in the after-effects of this disease, because the after-effects may be proof after all of the primary character of the disease process. The other day I examined a boy about 20 years

of age who had had scarlet fever 3 months before, and the outstanding lesion in his case was a thickening of the walls of the blood vessels, almost to the point of occlusion, in a great many organs, particularly the kidneys. This boy had developed hypertension, but had not developed glomerulonephritis. I am wondering if Dr. Smith has had any experience in the later stages of scarlet fever.

(Dr. Norbert Enzer, Milwaukee.) Several years ago we attempted to repeat and confirm Duval's experimental production of glomerular nephritis, and in the course of these experiments lesions were produced in rabbits similar to those exhibited by Dr. Smith. This was particularly true of the kidney lesions. We were unable to stain bacteria in the tissues, but did find bacteria in the blood stream. In these animals we frequently found hyaline thrombi and even cellular thrombi in the afferent glomerular vessels. Also in these animals we occasionally encountered medial necrosis of the medium sized arteries of the kidneys. These lesions were very similar to the early findings in periarteritis nodosa. I should like to know from Dr. Smith whether he found thromboses in the arterioles of the kidney, or evidence of medial necrosis in the vessels.

(Dr. Smith, closing.) Obviously one cannot say much very conclusively in 10 minutes about the details of the work which has been done on this. In reply to Dr. Moon, we have attempted in all of these cases to take cultures of the individual viscera and of the blood stream. In a small percentage of these we have had positive blood cultures. These slides have all been stained for bacteria by methylene blue or Gram's stain, and in no instance where these lesions have occurred have we been able to demonstrate organisms.

In reply to Dr. Mudd's question, the blood cultures ran about 20 per cent positive in these cases, but the visceral cultures have almost always been negative.

In regard to the question of this representing a true scarlatina picture or the results of late secondary complications, I think I can answer that best by saying that I tried to emphasize that in these cases the individuals nearly all died within the first week or 10 days of the disease. As a matter of fact, a number of the slides that I showed came from patients on the 3rd to 4th day of the disease clinically, so that we are dealing with a very acute process which we feel is fundamentally the basis of the scarlet fever infection rather than a secondary late manifestation.

There are occasional instances in the slides of the heart in which we have found fairly circumscribed lesions in the interstitial tissue, but in no instance have we found anything that we were willing to classify as a definite Aschoff body. I have tried to avoid any particular reference to the cardiac pathology because we are expecting to present a comparable study of the rheumatic and scarlatinal hearts at a later date with Dr. Louis Gross.

The endothelial injury we think is pretty clear-cut in these cases, as we have seen it in a good many hundred sections from the very onset of the disease. We found a definite degeneration of the endothelial cells lining the vessels. This is not necessarily either on the venous or arterial side. Both were involved. Perhaps there is a little greater emphasis on the venous side than on the arterial. Then following such damage to these cells we found the usual proliferative change of their vascular endothelium.

I am perfectly willing that Dr. Karsner should take exception to my rather broad statement of specificity because I think that I may have been stretching a point for the sake of emphasis. As far as we are concerned, in making examinations in cases of scarlet fever, diphtheria, measles, pertussis and varicella, and

also in the group of some of the virus infections, particularly poliomyelitis, we have found certain vascular changes which are perhaps in some respects comparable, but we never have seen in any of these other diseases any such definite relationship, with such clear-cut progressive stages in the development of these lesions. The changes are so characteristic that we have been able to make a fairly accurate tentative diagnosis of scarlatina on the basis of histology alone.

In regard to the after-effects, unfortunately all our cases are acute. We have very little opportunity for follow-up work, and we have had no opportunity, therefore, to know what happens to these individuals later in life. It seems to me very probable that there might be some relation between these acute changes and changes that occur later in the blood vessels.

It is quite true that some of these changes might easily be mistaken for acute periarteritis in the initial stage, and in perhaps a half dozen of our cases we have been able to demonstrate acute necrotizing lesions in the vessel walls. In such cases we have found positive blood and tissue cultures, so that we feel there may be some secondary relationship under these circumstances. Where the lesion has been what we might speak of as "Simon pure," we have not been able to demonstrate these acute necrotizing changes, nor to get positive visceral cultures. We have not seen the characteristic thrombi in the afferent capillaries in these cases, nor in our experimental animals.

I do not know just how important these blood vessel lesions may be in respect to late effects, in the kidney particularly, nor the portal infiltration described, to the development of a subsequent cirrhosis of the liver, but it seems quite reasonable that such extensive changes as we have demonstrated may well be significant factors in the pathology of the heart, the liver and the kidney in later life.

THE PATHOLOGY OF CHRONIC ULCERATIVE COLITIS. H. E. Robertson, Rochester, Minn.

Abstract. Chronic ulcerative colitis owes many of its peculiarities to its long duration with alternating periods of quiescence and repair and periods of relapse. Deep ulceration, even to perforation, extensive inflammation and scarring of the submucosa, hemorrhage, hypertrophy of the muscular coats, and irregular new growth of the epithelial layer constitute the usual pathological picture. Repair of the mucosa on a pathological submucosa often gives disorderly polypoid growths which tend to become carcinomatous. The frequency with which carcinoma complicates this disease, even in young adults, is an outstanding example of the effect of chronic inflammatory processes on the development of cancer.

Discussion

(Dr. Paul R. Cannon, Chicago.) I should like to ask Dr. Robertson what is his interpretation of these linear ulcerations, how frequently he observed them, and what he thinks of their pathogenesis.

(Dr. E. T. Bell, Minneapolis.) What is the incidence of bacillary dysentery in Dr. Robertson's experience, as compared with this non-specific colitis?

(Dr. Max B. Lurie, Philadelphia.) What do the blood vessels going into the polypi show? Do they show fibrosis?

(Dr. Robertson, closing.) Replying to the last question first, the blood vessels going into these polypi show no particular changes.

There has been, so far as I am aware, no instance of the bacillary type of dysentery. In about half the cases the organism described by Bargaen has been isolated in pure culture.

The longitudinal ulcerations I think come about by the contraction of the colon in which a part of the mucosa is protected.

A PRELIMINARY REPORT ON *INTRA VITAM* BIOPSY STUDIES OF THE PATHOGENESIS OF PNEUMOCOCCUS LOBAR PNEUMONIA. T. J. Curphey, Brooklyn, New York.

Abstract. Biopsy sections of the lungs of patients suffering from lobar pneumonia were obtained from a series of recovered cases at different stages of their disease as well as from cases at postmortem in which autopsy was not permitted. The method used was the punch biopsy of Hoffman. The object of the study was to determine whether the histogenesis of this process differed in recovered cases from that usually seen in postmortem studies. From the material thus far available, certain definite changes can be noted in the pericapillary histiocytes in the early stages of the disease, which would suggest that these cells play a definite defensive rôle. Certain interesting changes are similarly noted in the alveolar capillaries. These preliminary observations tend to stress the need for further *intra vitam* studies, in more cytological detail, of the mesothelial elements of the lung in lobar pneumonia.

Discussion

(Dr. Arthur W. Wright, Albany.) I have two questions I should like to ask Dr. Curphey. To me the introduction of a trocar into an infected focus in a lung seems a rather dangerous procedure. I should like to ask how often after the biopsy specimen has been taken from a pneumonic lung secondary infections such as acute pleuritis, which perhaps later developed into empyema, have occurred, and whether or not there is serious danger of such secondary infections. In the second place, after seeing the photomicrographs which were thrown on the screen, I should like to ask if the method as used so far has shown anything that has not yet been demonstrated in pneumonic lungs obtained at autopsy. Many of us would like to know more than we do about the pathogenesis of lobar pneumonia and this method may offer a means of studying the early changes that occur in this disease, but I fail to see that as yet anything new has been learned. In my opinion this technique is not only too dangerous for the patient, but at present seems to offer too little in the way of added knowledge of the pathology of the disease to justify its use.

(Dr. Eugene L. Opie, New York City.) I have been interested in secondary infections that occur in association with pneumonia, particularly when it follows influenza, and I have been impressed by the readiness with which *Streptococcus hemolyticus* invades a preëxisting lobar pneumonia. In the presence of secondary infection with *Streptococcus hemolyticus*, abscess formation and empyema might follow the introduction of a needle large enough to remove lung tissue.

(Dr. Curphey, closing.) In answer to Dr. Wright's question, I may say that we do not think the incidence of empyema is any greater in the group biopsied than in a series of non-biopsied cases, based of course on physical findings and X-ray evidence. The point we are trying to make about the use of this method is that we realize the risk, of course. I stressed that. We feel very definitely that

the pathology of recovery of lobar pneumonia cannot be studied on the autopsy table. That was the prime motive for instituting this method.

I think Dr. Opie's point is well taken. I unfortunately am not able to give him any definite answer; I cannot say how frequently streptococcus infections follow these cases.

PULMONARY CHANGES DUE TO THE ASPIRATION OF LIPIDS AND MINERAL OIL.
Irving Graef, New York City.

Abstract. Since Laughlen's report (1925) and Pinkerton's clinical and experimental observations (1927 and 1928) of pulmonary inflammation associated with the deposit of oils and fats, an increasing number of instances of this condition have appeared in the literature.

We have studied 6 cases — 3 in infants (aged 6 months, 16 months, and 18 months respectively) and 3 in adults (aged 54, 66, and 70), in which oily material was demonstrable in considerable quantity in the lungs. In 4 instances an unsaponifiable oil (liquid at room temperature) was identified in the pulmonary deposits, and in 2 there was a mixture of fats and fatty acids.

Pulmonary suppuration was present in 4 instances. The presence of oily substances was anticipated in the lung on macroscopic examination twice. The lesions in the cases associated with mineral oil are strikingly similar in that the substance was dispersed in fine droplets, usually intracellular in location, and occupied the interstitial tissue at the expense of the alveolar spaces. In many instances lobular architecture was obliterated with a few rudimentary sacs as the only indication of respiratory parenchyma. The oily material in these cases was also found in intra-alveolar macrophages; multinucleated foreign body giant cells containing fat droplets were found in 1 case. In the same case there were macroscopic lesions which were mistaken for tumor metastasis at close examination (this patient had a recurrent adamantinoma of the mouth). The nodules were composed of dense fibrous tissue containing free globules of oil and a rich intercellular deposit which resembled adult adipose tissue. There was a marked accumulation of the offending substance around the blood vessels and bronchi. In the infants there was a diffuse increase in reticular and collagenous fibers around the distended oil-containing cells.

In 1 instance in which the aspiration of large quantities of neutral fat and fatty acids was inferred, there was striking necrosis of the lung resembling caseous necrosis in tuberculosis. An acid-fast membrane similar to the familiar hyaline membrane of other pulmonary lesions was demonstrated around air bubbles in the aspirated material. Another case associated with the deposit of neutral fat and fatty acids was found on reviewing a case of bronchogenic carcinoma with a broncho-esophageal fistula and multiple bronchiectatic abscesses with marked fibrosis in the appended lobe. In the walls of the cavities and in the proliferative interstitial tissue there were abundant deposits of fatty substances identified as true fats and fatty acids.

Lipid analyses were done on material from 4 of the 6 cases. The amount of total lipid, total unsaponifiable and saponifiable material, and the cholesterol content were determined in samples yielding large amounts of unsaponifiable material. Seven control specimens showing pneumonia, chronic passive congestion or no lesions were also analyzed. Identification of the unsaponifiable substance supported the histochemical examination in 2 of the 4 cases associated with mineral oil (there was not sufficient material in the third for analysis

and the fourth had been preserved in alcohol). In the case with massive aspiration of fat and free fatty acids, showing necrotizing pneumonitis with the peculiar formation of an acid-fast membrane, the saponification number and the iodine number were done and indicated that milk fat and the fatty acids of butter fat were probably present. A qualitative test for cod liver oil yielded positive results in this case as well.

Histochemical examination is an even more satisfactory method of identifying the offending substance, because it can be localized as well. Failure to reduce osmic acid or to react with Nile blue sulphate, the absence of anisotropic globules and a yellow stain with scharlach R serve to identify mineral oil. The reduction of osmic acid, the appropriate reaction with Nile blue sulphate, and orange red or salmon red reactions with scharlach R indicate the presence of a neutral fat or fatty acid. Formation of an acid-fast membrane indicates the presence of "blown" fatty acids described by Pinkerton in rabbits given oils rich in free fatty acid. The absence of anisotropic droplets rules out the presence of cholesterol or cholesterol esters. Solubility in lipid solvents may also be used.

Clinical data confirmed the use of the offending substance in considerable quantities in 5 of the 6 cases. Particular attention should be paid to the cases of mineral oil deposit because of the wide use of this substance as a vehicle for medication introduced in the nasopharyngeal passages and as an intestinal lubricant.

Mechanisms by which this substance gains entrance into the trachea and lung are not clear. Being non-irritating on the surface of the pharyngeal mucosa, it does not incite the cough reflex. The presence of mild anesthetic substances, like menthol in some preparations, may also enhance their passage into the trachea. Primary defective action of cilia in chronic infections of the respiratory tract, or the loss of the cough reflex in weak and debilitated individuals may also play a rôle.

Discussion

(Dr. Andrea Saccone, New York City.) This wonderful presentation by Dr. Graef has been corroborated by Roussy and Besançon, in the last number of the French Archives of Pathological Anatomy; in this article the authors are describing the possible pathology of lipiodol injected into the lungs. They emphasize very much that while vegetable oil does not produce any pathological lesions in the lung of the experimental animal, the mineral oils are responsible for extensive pathological conditions in the lungs.

(Dr. D. Murray Angevine, New York City.) I have had an opportunity to autopsy 2 of these cases at the New York Hospital. The first case was diagnosed clinically and at autopsy presented a characteristic picture; never having seen a case of oil pneumonia before, it was easily recognized when suspected. On scraping the lung tissue at the autopsy table oil droplets were readily found. The second case was not diagnosed clinically, but at the autopsy table, being aware from the appearance of the lung that some oily substance might be present, the unmistakable odor of cod liver oil was readily detected, showing that the sense of smell may be of value in making this diagnosis. Even after fixation in formalin one could detect the odor of fish oil. Several observers who had not seen the lung at autopsy examined it later and detected the odor of cod liver oil. Color tests for the presence of vitamin A were done.

I do not think that Dr. Graef has brought out the fact clearly enough that in most of these cases the individuals are in a debilitated condition. In our cases,

one child had been in a plaster cast for 10 months, and another child had a definite hydrocephalus with degeneration of the basal ganglia.

I should like to ask one question. One of your cases showed a large amount of fat apparently only in the mononuclear cells. We did frozen sections stained selectively for fat on several cases of uncomplicated bronchopneumonia, and were surprised at the amount of fat found in the mononuclear phagocytes in these cases. Was Dr. Graef's experience similar?

(Dr. Paul R. Cannon, Chicago.) I have studied 1 case of aspiration of cod liver oil which was of interest from the standpoint of the pathogenesis in that the trouble seemed to start from the forcing of the oil. The parents held the nose of the child and forced the child in spite of much resistance to take the cod liver oil, and the symptoms dated from that period. The lesions were more advanced than those shown by Dr. Graef in that three large cavities appeared in the lungs. These were demonstrated by X-ray before death.

In regard to the mechanism of the process in the lungs, I believe the mechanism may be much simpler than is usually supposed. I have taken normal rabbits and dropped a 50 per cent emulsion of cod liver oil into their nostrils, and have been able to demonstrate the oil in the alveolar spaces within 48 hours. Fischer-Wasels reported finding about 100 cc. of mineral oil in the lungs of an elderly woman dying with extensive pulmonary fibrosis. He found that this woman had been in the habit for 20 years of using mentholated mineral oil as a nasal spray and that she had bought it in large quantities from her druggist. It seems to me this subject is particularly important because of the extensive advertising over the radio and in the newspapers on the use of oil droplets for respiratory infections, and undoubtedly this condition has been much more common recently. We have had 6 cases which we believe were of oil aspiration, but did not study them particularly until we encountered this rather dramatic one of cod liver oil aspiration.

(Dr. Alan R. Moritz, Cleveland.) I should like to ask Dr. Graef if fat stains on the mediastinal lymph nodes give any indication that the oil was being mobilized.

(Dr. E. T. Bell, Minneapolis.) Dr. Graef's photomicrographs bring out a point which a great many of us have no doubt seen, the fact that the alveoli are lined by columnar epithelium. I should like to ask if he has studied the origin of these cells. There is quite a body of opinion among histologists that the lung alveoli have no epithelial lining, yet in these cases of lipoid pneumonia nearly everyone finds this appearance: the alveoli lined by typical epithelial cells, usually columnar.

(Dr. Stuart Mudd, Philadelphia.) In the course of direct observations on *in vitro* phagocytosis Mrs. Mudd and I have seen rather striking differences between the macrophages and the polymorphonuclear leukocytes in that the macrophages readily ingest mineral oil, and the polymorphonuclear cells do not. I take it that Dr. Graef found the mineral oil in macrophages. Did he observe it in the polymorphonuclear leukocytes, and if not, was the same difference between the two types of phagocyte observable with other types of oil?

(Dr. Alfred Plaut, New York City.) I wonder whether the distribution of the oil might partly be merely a physical process. Oil diffuses on dry surfaces. When we put oil in a container, we will find that it climbs up and the next day oil will be found on the outside also. I do not know whether on a wet surface oil can be diffused, but it might be interesting to look for oil in the accessory nasal sinuses.

(Dr. Mudd.) Oil cannot spread over a wet surface. However, it may be the trachea under certain circumstances is not so wet; I do not know.

(Dr. H. Edward MacMahon, Boston.) From Dr. Graef's paper one might be led to believe that whenever a group of alveoli filled with lipid-containing cells is found, this material must be of exogenous origin, reaching the lung by aspiration. I should like to point out that it is a fairly common observation, both in gross and microscopically, to find large and smaller areas of lung parenchyma adjacent to chronic suppurative lesions in the lungs and especially in the neighborhood of a malignant tumor in which the alveoli and also, though to a less marked degree, the interstitial tissue are rich in large, swollen, desquamated epithelial cells and mononuclear cells filled with lipid. Such findings are extremely variable, at times occupying wide tracts of lung tissue, and resemble so closely the lesions described in this paper as to be indistinguishable from them. In so far as Dr. Graef and others who have discussed this paper have made no mention of the possibility of an endogenous source of lipid material, namely, from retention, degeneration and a disintegration of cells within the lung, I should like to emphasize the importance of the endogenous source of lipoids in contrast to the exogenous material obtained by aspiration. I believe the former source to be equally important in the etiology of lipid pneumonia, and in adults, at least, the more common.

(Dr. Norbert Enzer, Milwaukee.) In only 1 case shown by Dr. Graef were multinucleated giant cells found. This, I believe, was in an adult with esophageal bronchial fistula and a chronic interstitial fibrosing pneumonia. It will be interesting to know whether Dr. Graef had observed giant cell formation in the children; also whether the fat surrounded by the giant cells was the same as that phagocytized by the macrophages.

(Dr. Kornel L. Terplan, Buffalo.) From some of the pictures Dr. Graef showed, it seems as if atelectasis has been one important pathogenetic factor, following the occlusion of bronchioli by the aspirated oil.

In the 1 case that I saw at the Buffalo Children's Hospital, the gross picture of the lungs was very characteristic. There was a distinct yellow color to the pulmonary parenchyma shining through the pleura. Appearance and consistency resembled more that of a chronic atelectasis with induration than a real pneumonia. The color was a peculiar mixture between the dark blue of atelectasis, and chrome yellow; it was entirely different from the color of lipid-granulation tissue, as seen in chronic pneumonia.

(Dr. Graef, closing.) The first point, in reference to the relative safety of the use of lipiodol in visualization of the bronchial tree, is that there is abundant evidence in the literature indicating that iodized vegetable oils produce practically no lasting pathological changes in the lung.

With reference to Dr. Angevine's remark about debility predisposing to this type of pneumonia, it has been the experience at Bellevue that aspiration pneumonias in general occur in debilitated and weak children. Nevertheless, in our 6 cases, 2 of the adults and 1 child were not debilitated or congenitally defective. Furthermore, in cases reported by other observers, Pinkerton particularly, it is worth knowing that some cases may show the clinical picture of an acute pulmonary infection, and only at autopsy may the presence of an oil or lipid material be noted. An example of this sort may be anticipated when massive aspiration of pharyngeal contents takes place at one time.

The finding of very occasional macrophages containing fat droplets in sections

from ordinary pneumonia is not unusual — especially in infants who may aspirate from their milk diets as a terminal or incidental event.

In reply to Dr. Moritz's question, occasionally macrophages in the lymphatics and the septa could be found containing oily material, but the amount of deposit in the mediastinal lymph nodes was extremely small and discovered with some difficulty.

We have also noted that the oily material was never deposited within polymorphonuclear leukocytes. When intracellular it was always in macrophages.

In reply to Dr. Bell's question, we have not systematically studied the origin of the lining cells of the alveoli in these cases.

As to Dr. Plaut's remarks in regard to the climbing phenomenon exhibited by oily material, I might mention that during our chemical analyses some of the recovered oil was left in a beaker overnight, some of which was found on the outside of the beaker the next morning.

The lipids found in cases in which true fats or fatty acids are demonstrated with careful histochemical methods may be endogenous (the use of scharlach R alone, of course, is not differential). Certainly in the 1 case of bronchiogenic carcinoma in which we found lipids in the walls of abscesses and supporting tissues, we realized that some of this material might have come from necrotic tumor. I make no claim as to its source, but wish to point out that this patient had a broncho-esophageal fistula, was on a fluid diet rich in fats, and the lipid-containing lesions were found only in the lobe appended to the fistulous bronchus.

We examined incidentally the lungs in 6 other cases of bronchogenic carcinoma with the idea that when these patients had been bronchoscoped the operators may have used mineral oil as a lubricant. These cases showed no lipids or oil-containing lesions.

If an alveolus is found stuffed with what appear to be fat-containing cells and the material dissolves scharlach R, but fails to reduce osmic acid, and is unsaponifiable on extraction, a paraffin oil of exogenous origin must be implicated.

The question of the formation of the multinucleated giant cells interested us because we had expected from Pinkerton's report that such cells might be found in the juvenile cases in which cod liver oil or mineral oil were implicated. They were not found in these, and I was left with the idea that the 1 adult case that did exhibit the foreign body giant cells had had the process for a far longer period than the infants. This was true in Pinkerton's experimental mineral oil lesions.

In reply to Dr. Terplan, primary atelectasis was not noted. What he termed bronchioles, I think, were non-muscular tubes which I believe represent cross-sections of alveoli with swollen lining epithelium. This formation is due to the increase in interstitial contents at the expense of the alveolar air space.

STUDIES IN EXPERIMENTAL OLEOTHORAX. D. H. Saley (by invitation), H. S. Willis and (by invitation) Lucia Ellwart, Northville, Mich.

Abstract. Several indications for the use of gomenolized oil have been given by clinicians who have used this product in the treatment of pulmonary and pleural tuberculosis. The results of the clinical use of oleothorax show considerable diversity and there is need of further knowledge of its effect.

This experiment was planned to ascertain the action of certain oils when injected intrapleurally in rabbits. Over 100 animals have been used. Paraffin and

cotton-seed oil alone and with gomenol were used in varying dosage. Efforts were made to determine if oil migrated from the pleural space, and if so, by what mechanism. Tieman's soluble blue was added to the oil as an emulsion and the retromanubrial lymph nodes, parietal pleura, lungs, diaphragm, liver and spleen were examined in gross and microscopically at varying intervals for evidence of the dye. Efforts were made to prevent the formation of adhesions by the oil by injecting amniotic fluid.

Pneumothorax was established in normal rabbits and oil introduced into the space, in some instances in one dose; in others the oil was given in weekly doses for 5 or 6 weeks.

The early response was the same to all oils introduced and consisted of a rather marked outpouring of fibrin which was deposited on all pleural surfaces. Soft, fibrinous adhesions were seen as early as a week after injection, and later these became firm, dense and widespread. In some specimens the heart and lung were glued to the sternum and thoracic wall by dense, massive adhesions. The diaphragm on the injected side showed typical elevation and thickening. Occasionally the retromanubrial lymph nodes became enlarged.

Cotton-seed oil tended to be rather quickly absorbed, while paraffin oil persisted in the pleural space indefinitely. Cotton-seed oil tended to form an emulsion with the fibrinous exudate, and the adhesions which it caused tended to become less dense as the oil was absorbed. Neither the amount of oil injected nor the proportion of gomenol used modified the final pathological finding; one injection caused practically the same effect, in regard to adhesions, as repeated injections.

Smears and cultures from material obtained intrapleurally were repeatedly sterile and one specimen with blue in the oil showed the retromanubrial nodes sacculated with oily pigment.

It was thought for a while that "Amfetin" facilitated absorption of adhesions, but further work made this highly problematical.

Histologically the area of reaction consisted of fibrin which embraced globules of fat and a few polymorphonuclear leukocytes and lymphocytes, giving the impression of granulation tissue. Later the cells became increased in number and finally the areas of reaction assumed a definitely more fibrous appearance. In instances where cotton-seed oil had been absorbed the adhesions and exudate underwent more or less absorption.

Discussion

(Dr. Andrea Saccone, New York City.) The Doctor reported that before the injection of oil into the pleura some air had been injected, and in the slide projected there was some injury to the alveolar wall. I should like to ask if any of the pathology of the alveoli could be produced by the previous insufflation of air in these cases.

(Dr. Howard T. Karsner, Cleveland.) I wish to confirm Dr. Willis' observation by observations I made in the course of studies of pulmonary infarction where active hyperemia of the lung was produced by the injection of mineral oil into the dog's thorax. No air was injected at that time; oil was placed in the lung, and the type of acute inflammatory change corresponds very closely to that shown by Dr. Willis. There was a good deal of phagocytosis of oil droplets, so that macrophages appear in the lymphatics under the pleura.

(Dr. Arthur J. Vorwald, Saranac Lake.) I also wish to confirm Dr. Willis' experiments. Dr. Hayes of Saranac Lake submitted tissue from experimental animals in which he injected the pleural cavity with paraffin oil, gomenol and olive oil to me for examination. In all instances, on examining the animals within comparable periods, he saw no difference in the type of reaction of various oils, and in all cases there was a marked thickening of the pleura, with very little penetration of the lung tissue by the oil.

(Dr. Willis, closing.) In reply to Dr. Saccone's question about the effect of air alone, I should like to say that the lung was collapsed by air being put into the thoracic cage rather than into the lung itself, and that when the lung was put down by air and oil added to the space the air was then withdrawn so that nothing more than a very transient exposure to the air existed. We did not check carefully the effect of the air alone, because the animal's response to pneumothorax is perfectly well known.

OBSERVATIONS ON THE VOLUME-DIAMETER RATIO OF ERYTHROCYTES IN SOME DISEASES. Theodore R. Waugh, Montreal, Canada.

Abstract. Observations are reported on changes in the shape of the human erythrocyte in various conditions, particularly the anemias. This alteration in shape is disclosed by comparison of the average corpuscle volume, average corpuscle diameter and an index of the thickness as determined by the formula $V/R^2 = \pi h$. In posthemorrhagic anemias and hypochromic anemia with achlorhydria the cells become smaller and thinner. In pernicious anemia there is a general tendency to a larger but flatter cell, though some cases show an abundance of small thick forms. In familiar hemolytic jaundice the erythrocytes are exceedingly small and thick (spherocytes). In a rare combination of hemolytic jaundice and pernicious anemia, this spherical character of the cells was preserved during reversion to the early embryonic, megaloblastic type.

Discussion

(Dr. Willard S. Hastings, Philadelphia.) I should like to ask Dr. Waugh whether he measured the cells in dry smears or wet mounts.

(Dr. Waugh.) These smears were made dry and stained in the usual manner. As I stated, I appreciate that the figures which one obtains in dry smears are by no means the same as one obtains in wet preparations. At the same time, we feel that the figures are representative of the change as they would be if one used the wet preparation. I emphasized the importance of picking out carefully certain places in the smears, because if one employs thick smears the results of course will be quite erroneous, but if thin smears are used and proper places picked out, one can measure 250 cells in one smear and 250 in another and get approximately the same figure for the average diameter reading.

STUDIES ON THE CELLULAR PATTERN OF BONE MARROW AT ROUTINE AUTOPSY. Robert J. Williams, Providence, R. I.

Abstract. This study was undertaken in view of the lack of available details concerning the cellular pattern of bone marrow at routine autopsy, using the section technique. In addition it is purposed to emphasize certain facts concerning the distribution of the red marrow in the long bones.

The material consists of marrow from the lumbar vertebra, sternum, the junc-

tion of the lower and middle third of the humerus, femur and tibia from 100 unselected cases in adults. Obvious diseases of the bone marrow were excluded. Cases with leukocytosis and secondary anemia were not excluded. Selection of these bones is based on Seecof's work which will be referred to later. The marrow from each bone was classified grossly as no hyperplasia, slightly hyperplastic, moderately hyperplastic and hyperplastic. This gross classification corresponded approximately to the marrow being fatty, being one-third red marrow, two-thirds red marrow, and being made up entirely of red marrow. The hyperplasia was confirmed microscopically.

The microscopic technique used was essentially that suggested by Custer with Maximow's hematoxylin-azure II eosin staining method. In the case of the vertebra and sternum, however, marrow pulp was placed on squares of paper and treated similarly to curettings, differential counts being made subsequently in small areas here and there where the histology was preserved.

In 50 consecutive cases, excepting those that were discarded because of unsatisfactory preparations, differential counts on 500 cells were done in the areas of maximum cellularity of the marrow from each bone classified as slightly hyperplastic or more. It is to be emphasized that as a result of this selection of areas of maximum cellularity the differential counts apply only to at least fairly well advanced hemopoiesis. Maximow's classification of the cells was selected, the purpose being to obtain a definite anatomical grouping of the cells rather than to adhere to any particular idea of histogenesis of the cells.

In the 100 cases, the presence and absence of red marrow in the long bones occur in orderly combinations. When hyperplasia is present in the tibia, it is also present in the femur and humerus. This combination occurred in 2 cases. It may be present only in the femur and humerus. This distribution of red marrow occurred in 31 cases. Hyperplasia may be present in the humerus alone, which occurred in 25 cases in this series. In the remaining 42 cases no hyperplasia in the long bones occurred.

Seecof in unpublished observations states: "Hyperplasia of the marrow in the long bones does not take place uniformly. It appears first in the humerus, then in the femur, last in the tibia . . ." — and he states further, "Moreover, when recession sets in the hyperplasia disappears first from the tibia, then from the femur and last from the humerus." The data presented are in agreement with Seecof's concept.

The correlation of the distribution of the red marrow in the long bones with age shows that with advancing years there is an increasing tendency for the marrow of the long bones to be fatty. For example, in the fourth decade, 1 case out of a total of 13 showed no hyperplasia in the long bones; in the sixth decade 8 cases out of 20 showed no hyperplasia in the long bones; in the eighth decade 9 cases out of 12 showed no hyperplasia in the long bones. Custer has recently emphasized the decreasing cellularity of the marrow in different bones with advancing years.

The differential counts on the marrow of the different bones in the same case were essentially the same. The maximum variation occurred in the case of the neutrophilic myelocyte, 7 cases showing a variation of over 10 per cent, the maximum being 19 per cent. Therefore, for the series it is fair to represent each case by an average of the percentage values of each cell type in the different bones of the same case. This was done and the data for each cell type were arranged in a percentage frequency table from which the range of average was calculated. For this series of cases the range of average for each cell type is de-

fined as the limits in percentage values within which 80 per cent or more of the cases occur.

The range of average for the neutrophilic myelocytes is from 25 to 45 per cent; that of the segmented cells and metamyelocytes from less than 1 to 6 per cent each, and that of the promyelocytes 1 per cent and less. The arithmetical averages are 32 per cent, 3 per cent, 4 per cent, and 0.5 per cent respectively.

The line of division between the myelocytes and the promyelocytes was arbitrarily drawn between those cells with the cytoplasm more than half filled with the specific granules, and those cells with the cytoplasm less than half filled with the specific granules. Therefore, it is seen that there is a noticeable lack of transition forms, *i.e.* the promyelocytes, between the immature basophilic stem cell, the hemocytoblast and the more mature form, the myelocyte.

The range of average for the polychromatophilic erythroblast and the normoblast is from 5 to 25 per cent each; that of the proerythroblast 1 per cent and less; that of the total number of cells of the erythrocytic series 20 to 45 per cent. The corresponding arithmetical averages are 13 per cent, 17 per cent, 0.3 per cent, and 29 per cent. Here too, there is a noticeable lack of transition forms between the hemocytoblast and the more mature erythroblast.

The range of average of the small lymphocytes is from 5 to 20 per cent, that of the plasma cells from less than 1 to 7 per cent, and that of the medium size lymphocytes 2 per cent and less. The corresponding arithmetical averages are 13 per cent, 4 per cent, and 0.7 per cent.

The extreme limits of dispersion of the hemocytoblasts are from less than 1 per cent to 3 per cent; that of the eosinophilic granulocytes from 1 to 8 per cent; that of the megakaryocyte from less than 1 to 2 per cent; that of the reticulo-endothelial cells from 2 to 8 per cent. The corresponding arithmetical averages are 0.8, 4, 0.5 and 4 per cent.

There was one exception in the case of the eosinophilic granulocytes not included in the data and the classification of the reticulo-endothelial cells is at variance with Maximow's classification. The unclassified cells were 5 per cent and less.

Maximow has described as characteristic of homoplastic hemopoiesis the hemopoietic pattern in which there is a lack of a significant number of transition forms between the hemocytoblast on one hand and the myelocyte and erythroblast on the other, the latter two type cells furnishing the adult granulocytes and erythrocytes of the blood. On this basis hemopoiesis was of the homoplastic type throughout in this series.

The average range of variation of the various type hemopoietic cells in material from routine autopsy has been shown.

It is believed that these data are of value in the study of the pathology of the bone marrow.

Discussion

(Dr. David P. Seecof, Montreal.) I want to congratulate Dr. Williams for undertaking a study of the cytology of the marrow in the different long bones. At this time, since he mentioned the unpublished observations I have made, it might be advisable briefly to point out some of the important practical facts that have been accumulated since I began examining the marrow of more than one long bone at autopsy back in 1920. The need for such an examination became apparent while performing an autopsy on a patient who died during a megaloblastic crisis, in whom during life up to 40 per cent of the circulating red

cells were nucleated. When the tibia was opened, I found that the marrow was completely fatty or resting and the question arose — where did the circulating nucleated red cells come from? The marrow of the femur showed a slight hyperplasia, but in the humerus the marrow was markedly hyperplastic. A search of the literature revealed that it had long been known that the marrow of long bones differed from that of flat and tail bones in mammals and in birds. It appeared from the biological and zoölogical literature that in the *normal* adult the usual demands for hematopoiesis were supplied by activity of the marrow of the flat bones and that the marrow of the long bones did not manufacture red cells unless there was a call for increased hematopoiesis, as in anemic states. I called the attention of Dr. Francis Peabody to this fact and he agreed on the fallacy of taking tibial punctures in studying the condition of the bone marrow in pernicious anemia. Since 1920, examination made on the long bones and the flat bones in over 1000 autopsies led to the following conclusions: If the marrow in the tibia, as also Dr. Williams' data showed, is resting or aplastic it does not mean that there is no hyperplasia in the other long bones. On the other hand, if the tibial marrow is hyperplastic, there is no need for examining the other bones, since they will all show hyperplastic marrow. This is of practical importance in all intravital bone-marrow studies. The fact that the long bones are not normally forming red cells suggests that if an *intra vitam* biopsy is desired for the study of the bone marrow it should be taken from the sternum, because of the more accessible bones; the sternum is always in active hyperplasia, whereas the tibia may or may not be. Similarly, if the problem is to determine if increased activity or hyperplasia of the marrow is present in the body, then the tibia is the bone to be examined. These facts seem to hold for all ages, as Dr. Williams pointed out. I have yet to see a case of aplasia of the marrow of the flat bones. The condition called aplastic anemia, in my opinion, does not exist, because I have seen many cases of pernicious anemia before liver therapy was instituted, *i.e.* before 1924, in which the marrow in the long bones was entirely aplastic and that in the flat bones was hyperplastic. Aplastic anemia was probably often erroneously diagnosed because only the long bones were examined. In old age one rarely finds active hyperplasia in the long bones. However, I have never seen aplastic marrow in the flat bones at any age or in any clinical disease. This holds for agranulocytosis also.

(Dr. William Boyd, Winnipeg.) There is a very striking plate in the book on pernicious anemia by Davidson and Gulland which shows a large number of bones in the body removed at one autopsy. It represents in color the remarkable patchiness of the hyperplasia, and certainly emphasizes the great fallibility of examining only one bone, or only one or two places in a bone.

(Dr. Williams, closing.) I should like to add in closing that I did not mention, because of lack of time, that the remainder of the cell types, which I did not show, were counted, but I did not have time to present the data.

HISTAMINE AND LEUKOCYTOSIS. Virgil H. Moon and (by invitation) Marshall M. Lieber, Philadelphia.

Abstract. The intravenous injection of histamine phosphate into cats is followed by prompt leukocytosis, preceded occasionally by slight leukopenia. The subcutaneous injection of histamine phosphate into monkeys is likewise followed by leukocytosis. Histamine phosphate injected intravenously or subcutaneously in man produces a transient leukopenia followed by a moderate leukocytosis.

This is not so marked as in cats and monkeys. The increase consists chiefly of polymorphonuclear neutrophils. Following single injections the leukocytic count in cats and in man returns to normal in 24 hours; in monkeys the increase frequently persists 48 hours.

Discussion

(Dr. E. M. Medlar, Mt. McGregor.) There is one thing I wish to point out in regard to the leukocytic reaction and that is this — you cannot pay any attention to less than a shift of 50 per cent in the total count, nor less than 10 per cent in the differential count. Conclusions should not be drawn unless there occurs a shift greater than that mentioned. I have taken individuals and punctured their fingers in bed, walking around and at rest, sitting in a chair, at 5 and 10 minute intervals, over $\frac{1}{2}$ to 2 hours, and in those individuals I have found a shift as high as 50 per cent in the total count, and as high as 10 per cent in the differential count. Such a degree of shifting is of no significance except that it shows the leukocytes to be unevenly distributed in the circulating blood.

(Dr. Moon, closing.) The variations were much more marked in cats and in monkeys. The average increase in cats was over 100 per cent, and the increase seen in monkeys ranged between 200 and 1000 per cent. The evidence thus far in human subjects would not be significant, were it not accompanied by this marked evidence seen in the experimental animals. I believe that the experiments indicate another analogy between histamine and Sir Thomas Lewis's H-substance which he investigated in human subjects. I would make a final suggestion that the form in which the histamine is present in the human case is not known, that is, the particular chemical combination in which it exists, and there is a possibility that that combination is more active physiologically than histamine phosphate.

STUDIES ON THE CHEMOTROPIC PROPERTIES OF POLYMORPHONUCLEAR LEUKOCYTES AND LYMPHOCYTES. Harold M. Dixon (by invitation) and Morton McCutcheon, Philadelphia, Pa.

Abstract. Chemotropism of human leukocytes was studied *in vitro* by the following method. A clump of bacteria is placed on a glass slide and dried; a drop of blood from the finger tip is lowered onto the bacteria and allowed to spread between slide and coverslip. This preparation is observed with the microscope at 37° C., and by means of a drawing ocular the path of each leukocyte is recorded at minute intervals. The net approach of leukocyte to bacteria is measured, and this distance is divided by the total path traversed in the same length of time. The quotient is taken as the measure of chemotropism and ranges in value from +1.00, when the leukocyte approaches the bacteria in a straight line, to -1.00 when it moves directly away. Polymorphonuclear leukocytes begin to move as soon as the preparation is warmed. All the cells in the same microscopic field as the bacteria display positive chemotropism, while in more distant fields the intensity of the reaction diminishes with the distance. When *Staphylococcus albus* was used as the source of attraction, no change in chemotropism was found over a 5 hour period; the width of the zone of attraction did not increase. With the highly pathogenic yeast, *Torula histolytica*, polymorphonuclears were at first attracted, but later some cells moved away in a nearly straight line, displaying negative chemotropism. No difference in rate of locomotion was observed between leukocytes that displayed positive chemotropism and those

moving at random in remote fields, that is, we observed no relation between rate of locomotion and chemotropism. In contrast with polymorphonuclears, lymphocytes were neither attracted nor repelled by *Staphylococcus albus* or tubercle bacilli. They were not attracted to caseous material from tuberculous man or chimpanzee. Blood lymphocytes from a case of acute lymphatic leukemia and from a case of infectious mononucleosis were not attracted by *Staphylococcus albus*. We have obtained no evidence that lymphocytes display chemotropism.

Discussion

(Dr. H. Gideon Wells, Chicago.) I should like to ask the relative speed of these different types of cells, lymphocytes and polymorphonuclear leukocytes.

(Dr. E. T. Bell, Minneapolis.) Is there any difference in behavior of the lymphocytes containing a small amount of cytoplasm as compared with those which contain a large amount of cytoplasm?

(Dr. Milton J. Grand, New York.) Were the bacteria grown in agar or in broth?

(Dr. Dixon, closing.) In answer to Dr. Wells' question about the speed of the cells, we found that the lymphocytes moved on an average of 13 microns per minute, while the polymorphonuclear leukocytes moved on an average of 30 to 33 microns per minute.

In reply to the question concerning the amount of cytoplasm of the lymphocytes, we have been unable to see any difference in the behavior of cells with relatively large amounts of cytoplasm and those with relatively small amounts of cytoplasm.

We have used both broth and agar and some of the tubercle bacilli were grown on protein-free medium. Also the organisms were washed in some instances, and still we found no difference in the chemotropic response.

THE MEGAKARYOCYTE IN THE CIRCULATING BLOOD WITH SPECIAL REFERENCE TO HODGKIN'S DISEASE. E. M. Medlar, Mt. McGregor, N. Y.

Abstract. It is generally recognized that the megakaryocyte is found in the circulating blood in various pathological conditions. It has been reported as present in cases of acute lobar pneumonia, myelogenous leukemia, polycythemia vera and in rare instances in Hodgkin's disease. A very rare case of megakaryocytic leukemia has been reported. In all of these reports the presence of fully differentiated megakaryocytes is what has been observed. The chief purpose of this presentation is to show the maturation phenomenon of the megakaryocyte from the marrow stem cell to the fully differentiated mammalian type of megakaryocyte as found in the circulating blood. In this maturation process there is a stage in which it is difficult and at times impossible to distinguish individual megakaryocytes with certainty from monocytes. In fact, the author believes that many cells which have been called monocytes in blood smears are megakaryocytes in the process of maturation. In other words, attention is drawn to the presence of immature megakaryocytes in the circulating blood. They probably occur much more commonly and in more pathological conditions than is at present recognized.

In regard to Hodgkin's disease, the author believes that immature megakaryocytes are a consistent finding in the circulating blood. In his study of blood smears from 20 cases of proved Hodgkin's disease they were present in all but two instances. In 5 cases where the author had an opportunity to examine

blood smears weekly over periods of months these cells have been present consistently. The number varied from 5 to 40 present.

Whether the presence of megakaryocytes may have a diagnostic significance in Hodgkin's disease is at present under investigation. From present data it would seem that if there is to be any diagnostic significance in the finding of megakaryocytes, it will have to be from the percentage and persistence on repeated examinations rather than on single observation.

The main distinguishing features between the monocyte and the megakaryocyte are: (a) the nucleus tends to be more complex and lobulated in the megakaryocyte than in the monocyte; (b), the cytoplasm of the maturing megakaryocyte is more granular and stains deeper with Wright's blood stain than does that of the monocyte; and (c), the immature megakaryocytes tend to have less cytoplasm compared to nuclear volume than do mature monocytes.

Discussion

(Dr. Virgil H. Moon, Philadelphia.) I recall some 20 years ago Bunting and Yates at Madison made blood counts in cases of Hodgkin's disease and described large mononuclear cells to which they did not apply a definite name cytologically, but it occurred in about 5 to 20 per cent in the blood examined in cases of Hodgkin's disease. I should like to ask Dr. Medlar whether in his opinion the megakaryocytes which he has found are identical with the cells described by Bunting and Yates.

(Dr. J. Furth, New York City.) It is to Dr. Medlar's merit that he has focused our attention on the megakaryocytes. This presentation brings up the question of the limitations of morphological studies for the interpretation of cytogenesis. The cytoplasmic fringes interpreted as evidence of ameoboid movement are possibly artefacts. Dried blood smears do not seem to be suitable to determine the identity and potentialities of the large mononuclear cells with basophilic cytoplasm. May I ask Dr. Medlar if he has studied these cells in the live state?

(Dr. Medlar, closing.) In reply to Dr. Moon's question, I sent blood smears from some of these cases out to Dr. Bunting and he said they were the same cells he had seen, but he thought they were monocytes and they had their origin in the bone. I agree that they come from the bone marrow and that they are monocytic in type, but I believe they are really megakaryocytes.

In reply to Dr. Furth's questions, I myself have not made any study of these cells in life. I may say in the case I showed which was diagnosed as monocytic leukemia the doctor who had the case studied these cells very carefully in supravital preparations and he found these cells moving all over the field, so there is no question in that case but that the cells are distinctly ameoboid.

As to their being artefacts, all I can say is that I have been as careful as I could be in selecting cells for demonstration to rule out as far as possible artefacts in the smear.

BONE CHANGES IN LEUKEMIA: PATHOLOGICAL FINDINGS. I. H. Erb, Toronto, Canada.

Abstract. The changes in bone in acute leukemia in childhood may be grouped under the following headings: (1) infiltration; (2) rarefaction; (3) proliferation; (4) degeneration, and (5) hemorrhage. These changes are illustrated in two

cases of leukemia, one that of a girl who died at the age of 6 years, the other that of a boy who died at the age of 2½ years.

Infiltration by leukemic cells may occur in bone as it does in the liver and kidneys or elsewhere and may involve the marrow cavity, haversian canals or subperiosteal regions. By replacing bone marrow it may give rise to profound anemia. It is not visible in roentgenograms. Rarefaction occurs chiefly toward the ends of the long bones, but may occur anywhere along the shaft and involve both cancellous and compact bone. Proliferation of new bone occurs underneath the periosteum following stripping up of the periosteum by infiltrating leukemic cells. This new bone formation, as well as the areas of rarefaction, are demonstrable by roentgenograms. Degeneration of masses of leukemic cells, as well as hemorrhage into the marrow cavity, may occur in the course of the disease, but are of little clinical significance.

BONE MARROW IN AGRANULOCYTOSIS. R. P. Custer, Philadelphia, Pa.

Abstract. Study of the bone marrow of 12 cases of idiopathic agranulocytosis showed a striking qualitative likeness. Clinically the cases presented either acute progressive or chronic continuous profound neutropenia, relative lymphocytosis (actual lymphopenia), no anemia or thrombocytopenia of consequence, and no hemorrhagic phenomena. Necrotizing mucosal lesions were more or less prominent. The uniform findings were as follows:

(a) Marked proliferation of myeloblasts which does not proceed at the expense of the other marrow elements and never results in the so-called "replacement anemia."

(b) Failure of these cells to mature, resulting in paucity of myelocytes and practically complete absence of segmented forms.

(c) Normal or slightly increased red blood cell formation.

(d) Slight hyperplasia of otherwise normal megakaryocytes.

(e) Infiltration of lymphocytes and plasmocytes with formation of folliculoid aggregations of these cells.

Degeneration and relative hypoplasia of the marrow were noted in 2 cases, although qualitative changes were similar to those in the other 10. Comparison of differential marrow counts from idiopathic and secondary agranulocytosis (arsphenamin and septic neutropenia) showed marked dissimilarity in that maturation of granulocytes was complete in the secondary types.

The following table attempts to link the various causes of neutropenia *per se* with changes in the marrow to afford the clinician better opportunity for classification of cases as idiopathic or symptomatic agranulocytosis:

I. With relatively "full" marrow, as result of:

(a) Severe toxemia (usually bacteria), through primary stimulation of granulopoietic tissue, then destruction of cells *in situ* or on entry into the circulating blood.

(b) The leukoses (leukemias), viz.:

(1) Aleukemic myelosis, through overproduction of granulocytes that either do not leave the marrow or are destroyed on entering blood.

(2) Lymphadenosis, through replacement of granulopoietic tissue.

(3) Reticulosis, through replacement of granulopoietic tissue.

(c) Idiopathic agranulocytosis (agranulocytosis of Schultz, agranulocytic angina, malignant neutropenia), through defective maturation of myeloblasts (most cases show full marrow; see II (e)).

II. With relatively "empty" marrow, as result of:

- (a) Severe toxemia (usually chemical), sometimes specific for neutrophils (benzol).
- (b) Marrow exhaustion, through protracted anemia, toxemia or infection.
- (c) Aplastic anemia (idiopathic), congenital or acquired.
- (d) Irradiation (roentgen ray or radium).
- (e) Idiopathic agranulocytosis (the occasional case).

The presence of a lesion of maturation specifically confined to the granulopoietic series, not reduplicated by diseases of known etiology, entitles idiopathic agranulocytosis to a place as a disease entity. Regarding the relation of amidopyrin it can hardly be deemed more than an exciting factor, certainly not the only one; the disease may prove to be a proliferative allergic condition with several precipitating agents.

Discussion

(Dr. E. T. Bell, Minneapolis.) I should like to ask Dr. Custer if he can give us the proportion of myeloblasts in the marrow of untreated pernicious anemia; also if these differential counts were made on smear preparations of marrow.

(Dr. David Seecof, Montreal.) I should like to ask if the same long bone was studied in all the cases. As I pointed out previously (in discussing Dr. Williams' paper), it would be unfair to make comparisons otherwise. Incidentally, the last table of Dr. Custer's data again raises the important question in relation to studies on the circulating blood and marrow changes, namely, what correlation is there between the findings in the circulating blood and the findings in the marrow of the bones? I found it is possible to have abnormal circulating blood findings in relation to myelocytes and erythroblasts and yet have the marrow of the flat bones yield negative findings, or that the marrow in long bones be resting or aplastic. At a given moment there may be no parallelism between the findings in the blood and in the marrow. I have worked for many years trying to find out what the time relations were between the appearance of abnormal circulating blood conditions and the appearance of abnormalities in the cytology of the bone marrow. I think in view of the fact that there is no correlation between the findings in the circulating blood in regard to the red cells and the myelocytes, at a given moment (or immediately before death), and the marrow in the long bones in particular, that it would be better to study the flat bones such as the vertebrae, sternum, ribs, and possibly the skull bones (where we find activity of the marrow all the time). The changes seen in the long bones may be those which have just been initiated. It seems to me that for these special cytological studies the marrow of the flat bones should be examined.

(Dr. J. Furth, New York City.) A characteristic feature of agranulocytosis is that it is not associated with anemia and as the studies of Dr. Custer show, erythropoiesis of the marrow is not disturbed. In the differentiation of acute leukemia from agranulocytosis, would it not be better to examine "reserve" marrow, *e.g.*, the marrow of the femur or tibia? Common agranulocytosis is regarded by Dr. Custer as an "idiopathic" disease different from that produced by known chemicals. Since recent studies have shown that long continued administration of chemicals such as amidopyrin produces similar changes in the marrow, is it not conceivable that the primary process is essentially the same in both conditions, namely, injury to the marrow, which fails to put out leukocytes in response to inflammatory irritants?

(Dr. George Shanks, Toronto.) I should like to call attention to the fact that there are a great many points of resemblance between kala azar in which there is a visible parasitic blocking in the hematopoietic system everywhere, and granulocytopenia with an active bone marrow. It seems to me that the study of kala azar, intensively, would shed a good deal of light not only on these problems but on the process of hematopoiesis.

(Dr. Custer, closing.) In answer to Dr. Bell's question, I do not recall with sufficient accuracy the differential counts on my pernicious anemia cases to give percentages of myeloblasts; I can only state that they do appear in the marrow in relatively few numbers, compared to megaloblasts. The differential counts were all done on bone marrow sections rather than smears; I believe that smears or imprints do not give nearly as accurate an index of the relative proportion of cells of the different series as does sectioned material. Counts on sections are more difficult, however, in that cytology may not be so clearly demonstrable as in smears, but I think that they can be accurately done.

The counts shown to-day were all made from the femur because I wanted to show the changes of early hyperplasia versus those of late, *i.e.*, possible qualitative differences between fulminating cases and cases of long-standing agranulocytosis. Qualitatively the counts on marrow from flat bones in these cases were very similar to those in the femur; had there been any significant variation, I should have mentioned it.

The point that Dr. Seecof made about early changes in previously fatty marrow differing from those in previously cellular marrow is true in the majority of instances; in other words, we often find hyperplasia in the adult femur to be of a type suitable to the demands of the occasion, if I may be permitted this teleological comment; adult femoral marrow will present predominantly myelocytic change in severe infection, erythroblastic in anemia. There is a reflected hyperplasia, however, in the various cell series not immediately concerned.

With regard to Dr. Furth's comments, I do not believe that the disease should be classed among the chemical toxemias, even though amidopyrin may appear to be one of the exciting causes; there is one qualitative change in the marrow that separates it from the former group, *i.e.*, a failure of maturation of the neutrophils: let us still call it idiopathic agranulocytosis. This maturation defect is not observed in other diseases and I think that we should still regard the condition, tentatively at least, as a disease entity.

POLYCYSTIC KIDNEYS. E. T. Bell, Minneapolis, Minn.

Abstract. Polycystic kidneys are found once in about every 500 postmortems, and from 5 to 10 per cent are unilateral. In our autopsy service about one-third of the cases occurred in infants, the majority of which were stillborn. There are relatively few clinical cases between infancy and the age of 25 years, but the disease is always congenital.

We may distinguish a surgical type in which the patient presents symptoms and signs referable to one kidney, *viz.* pain, tumor, hematuria, infection, and so on.

In the medical type the symptoms are those of acute or chronic renal insufficiency, and the functional disturbances correspond to those of contracted kidneys. Attacks of hematuria are, however, distinctive.

Edema is rarely prominent, and cardiac failure is unusual. The systolic blood pressure is 150 mm. Hg or higher in over 50 per cent of the cases that have been

reported and hypertension is somewhat more frequent in advanced than in early stages of the disease. Cardiac hypertrophy often develops but is much less pronounced than in primary hypertension. Retinal changes of the hypertensive type may be found especially in those with very high blood pressure. Some patients live many years after symptoms have developed. When the renal reserve is low, *i.e.* in advanced cases, pregnancy causes a typical nephritic toxemia, but there is no disturbance when the renal reserve is good.

There is abundant evidence that polycystic renal disease has a strong hereditary tendency.

The pyelogram is of great diagnostic value in cases where the diagnosis is otherwise difficult.

In the newborn group the outstanding structural changes are: the presence of numerous cysts, hypoplasia of parenchyma, *i.e.* a great reduction in the number of nephrons, and an excessive amount of interstitial connective tissue.

The numerous "glomerular" cysts are interpreted as vestigial structures derived from the first three or four generations of tubules.

In the subclinical group there is abundant renal parenchyma between the cysts; while in the clinical group the parenchyma may be reduced to a few small scattered islands.

The progressive atrophy of the parenchyma is brought about chiefly by continuous expansion of the cysts. Arterial disease plays a minor rôle in this process except in the occasional case in which true primary hypertension is superimposed on the cystic disease.

The arteries usually show a marked intimal thickening which is attributed chiefly to disuse atrophy but partly to hypertension. Medial fibrosis in the arteries is explainable on the basis of age.

The arterioles show no marked intimal diseases except when primary hypertension is a complication. However, they often show a marked medial fibrosis. This process is not true arteriosclerosis.

Kampmeier's theory of the origin of the cysts is favored.

One case is described (No. 44) in which compensatory dilatation of persistent tubules in a hypertensive contracted kidney caused it to resemble the true congenital cystic kidney.

Discussion

(Dr. George Baehr, New York City.) In the study to which Dr. Bell has very kindly referred, the arterial tree of polycystic kidneys was injected with a barium gelatine mixture and the kidneys then studied roentgenologically. We found that in advanced polycystic kidney disease most of the arterioles become obliterated and impermeable to the injecting fluid. As one would expect in any diffuse disease of the kidney, or any other organ, extensive arteriolar disease had occurred secondarily. Dr. Bell's observations that the vascular alterations are largely a medial disease are extremely interesting. In 1 case we have even encountered a necrotizing arteritis in the arterioles of the polycystic kidney which morphologically was identical with the type of disease described by Fahr and Volhardt as malignant sclerosis. The changes in the arterioles which occur secondarily in polycystic kidney disease must be at least a contributing cause in the production of arterial hypertension, renal insufficiency and ischemic sclerosis of the remaining small areas of renal parenchyma. This arteriolar sclerosis may even be complicated by the process which has been described as malignant sclerosis.

(Dr. Bell, closing.) I am inclined to think that this appearance of malignant sclerosis which Dr. Baehr refers to is an associated disease. I have 1 case in which there is a little arteriolar sclerosis of the ordinary type, and it is apparently a case of primary hypertension combined with polycystic kidney. Arteriolar sclerosis is so rare in polycystic kidneys that I think it is simpler to think of it as a combination of two diseases rather than as an arteriolar disease due to the polycystic kidney itself.

ANOMALIES OF THE CIRCLE OF WILLIS AND SERPENTINE ANEURYSMS OF THE INTERNAL CAROTID ARTERY: THEIR RELATION TO ENCEPHALOMALACIA AND CEREBRAL HEMORRHAGE. Otto Saphir, Chicago, Ill.

Abstract. A number of instances of encephalomalacia and cerebral hemorrhage were encountered in which a careful examination revealed the absence of occluding lesions of the vessels at the base of the brain. A morphological explanation for the encephalomalacia was not recognized until the circle of Willis was carefully examined and anomalies found, in some instances with resulting interruption of the circulation between the internal carotid and vertebral arteries. Anomalies of the circle of Willis have been frequently encountered since close attention has been paid to these vessels. In other instances occluding lesions were found in the internal carotid artery either in its petrous portion within the temporal bone or in its cavernous portion. These occluding lesions were the result of severe arteriosclerosis with formation of serpentine aneurysms and with production of ridges which completely occluded the lumen.

An interference with the passage of blood through the circle of Willis or occlusion of one internal carotid artery does not make itself manifest until the circulation through the other internal carotid artery, or both internal carotid and vertebral arteries, respectively, is impaired. The impaired circulation may be caused by an arteriosclerosis of the arteries of the brain or by a failing heart, evidence of which may be deduced from the findings of myocardial fibrosis and chronic passive hyperemia of the various organs. As a result of the impaired vis a tergo and arteriosclerosis of the vessels of the base of the brain, complicated by complete separation of the two arterial channels of the brain or occlusion of one of the internal carotid arteries, encephalomalacia may ensue. Before one resorts to an explanation primarily based on functional disturbances, three factors must be investigated:

1. The entire course of the internal carotid and vertebral arteries must be examined for occluding lesions.
2. The patency of the circle of Willis should be ascertained.
3. Morphological evidence of a failing heart must be sought.

Discussion

(Dr. Shields Warren, Boston.) I should like to ask Dr. Saphir if he considers these aneurysms to be of acquired or congenital origin. I judge from the tendency of association with other anomalies in the circle of Willis that the congenital origin is a very definite possibility. I have had 1 case of marked bilateral aneurysm of the anterior carotid also with absence of the posterior communicating branches in which a long course of neurological symptoms of a rather puzzling sort increasing in intensity suggested that the arterial change had been present from birth.

(Dr. E. Libman, New York City.) I want to make a clinical point in connection with this important communication of Dr. Saphir's, and that is that clinicians are not sufficiently in the habit of examining the abdominal aorta and the carotid arteries for evidence of arterial disease. It was pointed out, a long time ago, that it is possible clinically to detect a carotid occlusion.

Just the other day Dr. Saphir visited me and we had a case in the office of a patient who presented a carotid obstruction due to a rather large plaque in the wall. The artery showed only slight pulsation above and exaggerated pulsation below the obstruction. On the right side there was a smaller plaque, without any evidence of obstruction. In occasional patients such plaques are very tender, and while I do not want to draw any definite conclusion, I wish to add that several times cerebral accidents occurred within 6 months to a year after the observation was made.

(Dr. Norbert Enzer, Milwaukee.) Aneurysms of the type described by Dr. Saphir should not be confused with those of distinctly congenital origin. Seven cases of congenital aneurysm have come to our attention. These always occur in the circle of Willis and its branches, always in multiples, and always at the bifurcation of vessels. These aneurysms are subject to thrombosis or rupture. In either event, the clinical syndrome in the congenital aneurysms is more apt to be referred to the meninges; whereas the aneurysms of the type Dr. Saphir described are associated with deep-seated cerebral symptoms.

(Dr. Saphir, closing.) In our cases I do not believe that the aneurysm can be explained on a congenital basis, but rather because of the tortuosity of the vessels which I think is the result of a severe arteriosclerosis.

As far as Dr. Libman's comment is concerned, I firmly believe that these cases are overlooked not only clinically but also pathologically.

AIR EMBOLISM FOLLOWING INTRAVENOUS DRIP. Kornel L. Terplan and (by invitation) Carl T. Javert, Buffalo, New York.

Abstract. In a colored male, 36 years of age, resection of a jejunal ulcer was performed. Postoperative course was uneventful for 12 days; then nausea and emesis developed. Duodenal decompression and continuous intravenous drip of normal saline and 5 per cent glucose were therefore employed. The drip functioned well for 48 hours but an occasional interruption resulted when the patient removed the needle from the vein. A cannula was then ligated in the cubital vein and in some manner the adapter to the cannula was dislodged. It was estimated that 15 minutes elapsed before it was replaced. During this period no blood ran from the cannula; the intravenous fluid wetted the bedding. On replacing the adapter, the drip continued and 400 cc. of saline were given. Nine hours following the dislodgement of the cannula, the patient became increasingly restless, confused, irrational, dyspneic and pulseless and convulsions developed. He made several attempts to get out of bed. Death occurred 20 hours after the adapter was dislodged.

Autopsy was performed 5 hours after death. The right ventricle and pulmonary artery contained fluid blood on which floated pinkish red clots, foamy, sponge-like in appearance, with distinct gaseous bubbles on their surfaces. These clots floated in water. The right atrium contained crural clots without air. The foramen ovale was closed. The left ventricle was practically empty. The other findings comprised a huge peptic ulcer in the duodenum with no gross bleeding point; tarry stool in the intestine; no peritonitis; no thrombo-emboli in the lungs.

The brain was anemic and edematous, weighing 1450 gm. Smears and cultures of the air-containing blood clots were negative for bacteria. There was no post-mortem autolysis, and gas bacillus effects could positively be ruled out. Bacteria stains were entirely negative. Smaller branches of the pulmonary artery also showed air bubbles within their bloody content. In the frontal lobe and the island of Reil distinct focal necrosis with complete disappearance of ganglion cells in the third layer was found. These changes resembled the ischemic lesions as described in circulatory disturbances of varied etiology (anoxemia, insulin shock, fat and air embolism).

The unusual spongy appearance of the clots in the right ventricle could be explained only by the presence of air in the ventricle, which was included in the clots when coagulation took place. The clots in the right atrium did not contain air. Apparently this excluded any postmortal entry of air into the blood in the right ventricle. The right ventricle was dilated and flabby; the left ventricle contained neither blood nor clots. Careful gross and histological examination did not reveal any other cause of death.

Attempts to reproduce foamy clots artificially at autopsy by injection of different quantities of air into the jugular vein in cases where fluid blood was suspected were unsuccessful. The air was found in the large veins but no clotting had occurred, although 15 to 30 minutes were permitted to elapse before the heart was removed.

It is believed that air entered the venous circulation when the adapter was dislodged from the cannula. The aspiratory effect of the negative intrathoracic pressure, together with a lowered venous pressure which accompanied the ligation of the vein, were considered as instrumental factors in the entrance of air. It must be remembered that no blood escaped from the cannula when the adapter was out of place.

A GROUP OF CASES CHARACTERIZED BY SYSTEMIC VASCULAR ALTERATIONS AND ASSOCIATED FREQUENTLY WITH LUPUS ERYTHEMATODES AND ENDOCARDITIS. George Baehr, Paul Klemperer and (by invitation) Arthur Schifrin, New York City.

Abstract. Attention is called to a group of cases which are characterized clinically by irregular fever with a tendency to remissions, by involvement of synovial and serous membranes (arthritis), pericarditis, pleuritis, by depression of bone marrow function (leukopenia, thrombopenia, anemia), and by clinical evidences of vascular alterations in the skin, the kidneys and the other viscera. Twenty-three of the cases presented the skin and mucous membrane lesions of disseminated lupus erythematosus. With rare exceptions, the patients were young females, most commonly in the second and third decade of life. Blood cultures were negative.

The macroscopic changes in the internal organs at autopsy were chiefly located within the heart and kidneys. A terminal lobular pneumonia was often present.

Serous membranes were involved in 17 of the 23 cases of lupus erythematosus, pericarditis being present in 12 cases. In 13 of the 23 cases a coarse verrucous form of endocarditis was found upon the mitral or tricuspid valves. In 5 of these the parietal endocardium also showed lesions which conformed to the original description of Libman-Sacks (atypical verrucous endocarditis). In the 8 other cases there were smaller verrucae on the valves. Nine cases had no endocarditis whatever. Aschoff bodies were not found in 22 hearts which were carefully

studied. The kidneys showed multiple shallow depressions in 2 cases. Anemic infarcts were occasionally found in spleen and kidneys, and several cases showed emboli within pulmonary arteries.

Microscopic examination revealed conspicuous vascular lesions in the finer ramifications of the systemic and sometimes also the pulmonary circulation. They were found in the kidneys in 20 out of 23 cases. In the other organs the incidence of vessel changes was not as high. In 6 cases the vascular lesions were widespread in all the viscera. The skin showed similar vascular alterations.

Histologically, the vessel lesions represent a variety of changes: (1) Simple dilation of capillary beds in certain areas, as in the skin, with blood and serous extravasations. (2) Proliferative lesions of the lining endothelium of capillaries, arterioles and venules, associated with thrombi which often obstruct or occlude the lumen. (3) Degenerative and necrotizing lesions in the wall of such vessels, associated with thrombosis and sometimes with hemorrhage into the adjacent tissues. The severer lesions are especially conspicuous in the capillaries and arterioles of the kidney.

Because all three types were often found in the same case, they may be considered as stages of the same underlying morbid process.

The glomerular changes were especially conspicuous in 18 of the cases. The commonest and most characteristic alteration was a peculiar hyaline thickening of the capillary walls which is striking even in sections stained with hematoxylin-eosin. We have described this as the "wire loop lesion." It was present in 13 cases. Proliferative and thrombotic lesions of glomerular loops were frequent. In 2 cases the glomerular changes were sufficiently extensive to be called a true diffuse glomerulonephritis. In 3 cases the proliferative and necrotic process involved only a segment of a glomerulus, thereby creating a superficial resemblance to the embolic glomerular lesions of subacute bacterial endocarditis.

Isolated vascular lesions of similar appearance may at times be encountered in a careful histological study of persons who have died of any acute or chronic infectious process (Sigmund). Our group of cases is distinguished by the systemic distribution of the vascular lesions in various viscera.

Discussion

(Dr. E. T. Bell, Minneapolis.) I feel fairly certain, Dr. Baehr, that these "wire loop" lesions that you pointed out in the first few slides are thickenings of the capillary basement membrane. That thickening occurs almost constantly in eclampsia, in chronic forms of lipoid nephrosis, and in hypertension, and occasionally in various other toxic diseases, even in pernicious anemia. We have to look at the basement membrane of the capillaries as much as at the endothelial cells in understanding these glomerular changes. I think none of these so-called embolic lesions are embolic in the sense that they are infarctions. I think they are due to bacteria which grow and produce a thrombosis of the capillaries. It was originally the idea of Löhlein himself that these were thromboses. He later changed to the idea of infarction.

(Dr. E. Libman, New York City.) This contribution, to my mind, is a very significant one and has implications of a wide nature, into which I cannot enter today because of lack of time. However, I would like to say a few things, mainly in order to clarify what I meant by characterizing certain examples of endocarditis as "indeterminate." This designation was intended to apply to all the cases in which the etiology was not known, and in which there were no distinctive

clinical or pathological criteria. Furthermore, it was intended to convey the idea that further studies might demonstrate that at least some of them represented an unusual reaction to an already known etiological agent. From this comparatively large collection of cases, Dr. Sacks and I segregated a small number because they had something clinical and pathological in common. These we called atypical verrucous endocarditis. We realized that some of the other cases in the indeterminate classification might be found to belong to this group.

As regards the question of lupus erythematosus, we published 4 cases of atypical verrucous endocarditis, of which 2 had facial lesions. Dr. Gross put on record a fifth case of ours, in which there was a secondary infection by non-hemolytic streptococci (subacute streptococcus endocarditis). This case also had an extensive facial eruption. The then dermatologist to Mount Sinai Hospital, Dr. Hermann Goldenberg, stated that while these eruptions resembled acute lupus erythematosus disseminatus, they differed because atrophy, hyperkeratosis and desquamation were absent. It is, of course, possible that others might have included these eruptions in the category of lupus erythematosus. The etiology of this disease is unknown, so that we may well be dealing with an indeterminate disease, from the dermatological standpoint.

In our original publication some vascular lesions were described. Those demonstrated by Dr. Baehr are of a different order. They are entirely new for the description of the pathology of lupus erythematosus, and we consider them an addition to our knowledge of atypical verrucous endocarditis, and of some other cases in the indeterminate group. It remains for further study to determine whether or not the cases (or some of them) of endocarditis described here today, which do not correspond to what Sacks and I called atypical verrucous endocarditis, really belong in the same category. I understand that all these hearts will be examined by Dr. Gross, who in a remarkable paper published in 1932 described the pathological changes in the heart in atypical verrucous endocarditis and concluded that certain of them are pathognomonic of the condition.

It has been pointed out that there are cases that have vascular lesions and no endocarditis, with or without lupus erythematosus. Some years ago I made the suggestion, and Dr. Baehr has made the same suggestion, that there may exist cases due to the same cause or causes as atypical verrucous endocarditis, which have no endocardial lesions. It is possible that in such conditions the vascular lesions will be the characteristic feature.

The whole subject is of wide importance, not only as regards endocarditis and vascular disease, but also as bearing upon the relationship of dermatology to internal medicine.

(Dr. Benjamin Clawson, Minneapolis.) I should like to ask Dr. Baehr what the blood cultures showed in these cases. I cannot quite understand why this group of cases should be classed as non-bacterial and non-rheumatic. I should also like to ask Dr. Baehr just why the hearts are not classified as acute rheumatic endocarditis. It is true that Aschoff bodies were not found in the myocardium, but nobody who has reported a large series of cases of acute rheumatic endocarditis has found Aschoff nodules in all instances, so I cannot see how you would rule rheumatic endocarditis out. The vegetations in the pictures looked to me like the vegetations in acute rheumatic endocarditis. It is true that they extended down on the chordae tendineae which is sometimes found in cases of acute rheumatic endocarditis. Will you explain to us, Dr. Baehr, just why you decided that these hearts are not acute rheumatic endocarditis or subacute bacterial endocarditis?

(Dr. Paul Klemperer, New York City.) May I answer two questions — the question of Dr. Bell and that of Dr. Clawson? The remarkable thickening of the walls of glomerular loops which we have observed was not seen by us in any of the control material we studied, including lipoid nephrosis. There is no reaction for amyloid and no lipoid within the loops. The peculiar hyalinization of the loops resembles, superficially, the thickening one observes commonly in glomeruli of some cases of arteriosclerosis with particular glomerular involvement. But in our cases there was no fat in these tufts. Furthermore, these were young people without arteriosclerosis. One must remember that these lesions were found in young individuals from 12 to 30 years of age, and not in old individuals. In regard to the question of eclampsia, I must confess my experience with eclampsia is limited. Similar lesions probably occur in eclampsia, which is also a toxic disease. We believe these vascular alterations are of toxic origin.

In reply to Dr. Clawson's question, we have examined the hearts very carefully. I think the paper of Dr. Libman and Dr. Sacks proved the fact that the endocardial lesions which they described are not rheumatic. Such extensive lesions on the parietal endocardium do not occur in rheumatic endocarditis.

In regard to the small verrucous lesions which we observed in 8 of our cases, the question of a possible rheumatic origin was seriously considered. In the second heart which Dr. Baehr showed there is a chronic valvular defect on top of which fresh coarse verrucae are found.

Dr. Gross has carried out extensive histological studies on these hearts. I do not know whether one can rely on the histological examination of the valves alone for the diagnosis of rheumatic endocarditis. One has to take the myocardium into consideration. It must be significant that in the 22 cases in which the heart muscle was studied with great care, no Aschoff bodies or other evidence of rheumatic fever was ever found. For this reason we feel that the endocarditis is not rheumatic. Furthermore, identical vascular and glomerular disease was found in 9 cases in which the endocardium was normal.

In regard to the question of bacteria, in 20 of the cases repeated blood cultures were negative. No bacteria were demonstrable in crushing of the vegetations or in sections.

I agree with Dr. Bell that the loop necroses are not of embolic nature. They are probably caused by local thrombosis. In 1 case we can be certain that they are not embolic because there was no endocarditis on the left side of the heart. In another case there was no endocarditis whatever. For this reason we feel the loop necroses which resemble embolic glomerular lesions are local thrombotic lesions.

(Dr. Baehr, closing.) Twenty-three years ago we studied the Löhlein lesions of subacute bacterial endocarditis in fresh and properly fixed material. In the earliest glomerular lesions we demonstrated masses of bacterial emboli microscopically. The clumps of bacteria had been caught in a glomerular loop, thrombosis had then taken place, occluding the lumen of the loop and ultimately necrosis occurred. The embolic origin of the glomerular lesions of subacute bacterial endocarditis was confirmed by Fahr and accepted by Löhlein. The true embolic glomerular lesions of subacute bacterial endocarditis differ in one essential respect from the glomerular loop necroses which we observed in 3 or 4 of our cases of lupus erythematosus. In embolic glomerular lesions, the remaining non-embolized portion of the glomerulus is absolutely normal. In the condition which we are now reporting, the lesion is quite different. Although the necrosis of a glomerular loop may look superficially like the embolic lesion, the rest of

the glomerulus is always altered to a considerable extent. Also no bacteria can be demonstrated in the lesions.

The questions concerning the non-rheumatic nature of the endocarditis have been answered by Dr. Klemperer as well as can be done in these few minutes. The verrucae are free of bacteria and thirty-six blood cultures in 20 cases proved to be negative. Furthermore, the intensity and the widespread distribution of the vascular lesions in various viscera stamp this condition as something quite distinctive. These vascular lesions undoubtedly bear an important relation to the disease which the dermatologists have known for a good many years as lupus erythematosus disseminatus.

ARTERIOLAR CHANGES IN ESSENTIAL HYPERTENSION. Alan R. Moritz and (by invitation) Mary Ruth Oldt, Cleveland.

Abstract. This investigation consisted of a histological study of the walls and measurements of the internal and external diameters of over 10,000 arterioles and small arteries, supplemented by a study of serial sections of selected vessels from the skeletal muscle and gastro-intestinal tract of 38 control and 38 hypertensive individuals.

Thickening of the walls and expansion of the external diameters were characteristic of the hypertensives as a group, but these dimensional changes were not great enough in samples of 75 vessels to permit distinction between control and hypertensive individuals in 80 per cent of the cases. Over 80 per cent of the hypertensive and control cases could be recognized as such by the presence or absence of arteriolar sclerosis in an objective microscopic examination of one section of skeletal muscle without measurements.

The vascular disease in individuals with persistent hypertension appeared to begin in the smallest arteries and not only affected different arteries in the same tissue with varying severity, but affected varying segments of the same arteriole differently. Smooth muscle hyperplasia and medial degeneration characterized the process which progressed with increasing intensity from the larger to the smaller arteries. The primary change in the arteries appeared to be smooth muscle hyperplasia with superimposed segmental medial degeneration. The occasional finding of a dilated, thin-walled, degenerate artery indicated that hyperplasia was not invariably antecedent.

Discussion

(Dr. H. Gideon Wells, Chicago.) I should like to ask what the significance is of these measurements of the lumen of arteries after death. The fact that the lumen is practically the same in sclerotic and normal arteries would seem to me to indicate that the arteries contract as much as they can, no matter in what condition the walls are, and the difference is close to zero. We know from injections of arteries under normal mean systolic pressure that an artery may present a smooth lumen for a long distance, in spite of the fact that in that area are very extensive sclerotic plaques. The lumen is the same, where the sclerotic plaques are, and where they are not. I do not see the significance of these internal diameter measurements after death.

(Dr. E. T. Bell, Minneapolis.) This is an interesting line of study which Dr. Moritz has taken up. The difficulties, as he no doubt realizes, are very great, since we do not know accurately the changes that occur after death. The younger the person, the greater the contraction after the vessel is taken out of the

body. In a young person the contraction of a large artery is sometimes as much as 30 per cent after it is removed from the body. How much they contract in a piece of muscle we do not know, but they probably contract more in younger people than in older ones who have stiff arteries. Another thing which causes a variation is that there is a difference in the number of hours after death at which the tissues are fixed. I have found that in a few hours after death the lumen is much smaller than after 48 hours. There is a rigor in the muscle that passes off after 48 hours. These changes introduce factors which are difficult to control.

(Dr. Moritz, closing.) In reply to Dr. Wells I wish to say that we did not assume that measurements of lumen diameters gave absolute information as to the patency of vessels in life. It was necessary, however, to measure lumen diameters to determine the relative wall thickness of vessels. The fact that the walls of the smallest arteries were thickened in the hypertensives was regarded as significant. Although this thickening was a group characteristic for the hypertensives it was not useful in distinguishing between hypertensive and non-hypertensive individuals.

In reply to Dr. Bell it can be said that there was no significant variation in the relative thickness of arteriolar walls of the control group that could be related to age. The work just reported was preceded by experiments designed to show whether physiological states of vessels, rigor or varying technical methods in the preparation of tissues would affect the relative thickness in arteriolar walls. No significant effect could be related to any of these factors so far as comparing the mean wall to lumen ratio of vessels in one sample with another was concerned.

THE PATHOLOGY OF ADENOMA OF THE BRONCHUS. Coleman B. Rabin and Sylvan Moolten (by invitation), New York City.

Abstract. Since the report of 12 cases of polypoid adenoma of the bronchus by Wessler and Rabin in 1932, 9 additional cases have been observed at the Mount Sinai Hospital. These are now presented together with a pathological study of the original 12 cases for the following reasons:

1. The tumors are still considered to be extremely rare because only sporadic cases have been reported.
2. They present difficulty in histological diagnosis which has resulted in their being reported erroneously as malignant tumors. In other cases their benignity has been recognized on clinical grounds, but the pathologist has been unable to differentiate them from malignant tumors.
3. Definite pathological criteria are presented by which these adenomas may be differentiated from carcinoma.

Discussion

(Dr. William Boyd, Winnipeg.) The remarkably long clinical duration is characteristic of these cases. In a recent case of mine the patient had severe periodic attacks of hemoptysis for 24 years. The microscopic picture was much more benign in type than most of those we have seen on the screen to-day.

STUDIES ON THE MITOSIS RATE IN TUMORS OF SEVERAL MAMMALIAN SPECIES. Albert E. Casey, University, Va.

Abstract. A dependable method having a low coefficient of error was employed to determine the average number of mitoses per 1000 tumor cells in some 300 tumors of man, mouse, rat, rabbit and dog. The tumors included a wide assort-

ment of benign and malignant tumors of both connective and epithelial tissue types, and such conditions as Hodgkin's disease, lymphatic leukemia, and so on. The average rate of mitosis was 10 per 1000 for the 300 tumors with variations from 0 to 32 per 1000. In some 30 tumors the rate of mitosis in the metastases was compared with the rate in the primary tumor and found to be identical in every instance. In 15 of 16 recurrences averaging 1 year after removal of the primary tumor, the rate was also identical. In the heavily irradiated exception the rate was much higher than in the primary tumor. Tumors of laboratory animals were found to have the same range of variation (0-32) as the tumors of man. The transplantable tumors used had rates of mitosis of more than 12 per 1000 and were nearly all anaplastic in appearance, thus corresponding to the more malignant tumors of man. No malignant tumors except basal cell epithelioma (which averaged 2 per 1000) had rates of mitosis of less than 4 per 1000 and no benign tumor a rate greater than 4 per 1000. There seems to be a very sharp line of cleavage between benign and malignant tumors at about 4 per 1000. Tumors from young individuals or from the internal organs averaged a higher rate of mitosis than the tumors from the surface of the body or from old people. Sarcoma and carcinoma of the same grade had similar rates of mitosis.

Mitosis counts were made on a series of about 100 tumors upon which five year follow-ups were available. These included mixed tumors of the parotid, tumors of the breast and cervix, and a few sarcomas. The 25 individuals with tumor mitosis rates of 3 or less were living at the end of the 5 year period, whereas 80 per cent of the 40 individuals with tumor mitosis rates above 12 per 1000 were dead at the end of 5 years. Fifty-seven per cent of the remainder having rates between 4 and 12 were dead at the end of 5 years, the tumors of this group falling into the pathological grading of I and II. The tumors with rates of 12 to 32 correspond to grades II plus, III, and IV. When the mortality was plotted on the ordinate and the mitosis rate on the abscissa with a scale having ascending intervals of 1, 2, 4, 8, 16, 32, a symmetrical smooth S-shaped curve with a sharp ascent at 4 to 7 mitoses per 1000 resulted. This indicates a very high correlation between the rate of cell division in a tumor and its malignancy. The method should prove valuable in studies on the biology of tumors particularly in indicating the relative action of various agents on the constitution of the host or on the growth rate of the tumor cells. The method has seemed helpful in differentiating benign from malignant tumors; it very largely eliminates the present subjective error in the estimation of the number of mitoses and substitutes a mathematically accurate estimate of the growth rate in a given tumor, which growth rate usually remains constant through primary, metastatic and perhaps also the recurrent phases of tumor growth in a given individual.

Discussion

(Dr. Shields Warren, Boston.) Last week Dr. Casey was good enough to tell me about this and I had the opportunity to have one of my men run over these counts on various types of cells. While we all recognize that mitotic activity is roughly parallel to growth rate, I think it is a mistake to depend too much on it, and I was rather interested that two of the relatively high mitotic counts, in the region of 19 and 21, were on tumors of fairly low malignancy, and that in two basal cell carcinomas the counts were 11 and 13 per thousand respectively. In carcinomas of the breast, on the other hand, the count was around 7 and 8 per thousand, as Dr. Casey's was. I feel that the number of mitoses is only one of

many factors that have to be taken into consideration in activity and growth, and particularly in the prognosis of a tumor.

THE EFFECT OF TESTICULAR EXTRACT ON A TRANSPLANTABLE EPITHELIAL TUMOR OF RABBITS. Thomas T. Walker, Watertown, New York.

Abstract. Testicular extract was found markedly to increase the growth of the Brown-Pierce carcinoma of rabbits as compared to control tumors resulting from equal inoculations of the same material. In a series of 13 animals constituting three separate experiments, tumors in the skin and testicle averaged about 200 per cent greater than the corresponding controls. Testicle extract was administered intravenously, 1 cc. per day, starting in some instances after definite tumor growths had appeared. In one experiment it was also injected with the tumor cell suspension. Tumors resulted from each inoculation in the treated series and in one of the controls the tumor grew for a time and later completely regressed. The testicle extract used was prepared from rabbit testicles as described by Duran-Reynals.

THE SO-CALLED BRENNER'S TUMOR OF THE OVARY. Stanley P. Reimann and (by invitation) Clark E. Brown, Philadelphia, Pa.

Abstract. A mixed Brenner tumor of the ovary occurring in a 52 year old woman was recorded. Emphasis was laid upon the biological and morphological differences between these tumors, which have had no notable endocrine activity, and the granulosa cell tumors of the ovary. The inadvisability of the term folliculoma, with which they have been designated previously, was mentioned. Brenner tumors have been discussed in the German literature at some length: Plaut, 9 cases, Meyer, 21 cases. The latter has pointed out their frequent coincidence in the same mass with pseudomucinous cystomas and has shown actual communication between the indifferent epithelium of the Brenner type and cysts lined with typical pseudomucinous epithelium. An etiological relation between the two has been suggested by Meyer's demonstration of pseudomucin-like cells in the strands of Brenner epithelium. The origin of these tumors is thought to be ovarian germinal epithelium in some postovogenic phase of its development, either as congenital foci of Walthard, or as later invasions of the epithelium into ovarian substance.

Discussion

(Dr. Harry C. Schmeisser, Memphis.) Dr. J. M. Maury and I reported a case of bilateral ovarian tumors of the Brenner type in the *American Journal of Obstetrics and Gynecology*, 1934, 27, 290-293. Dr. Robert Meyer of Berlin, who is an authority on this tumor, confirmed the diagnosis. The solid type of Brenner's tumor occurred in the right ovary, which was converted into a very firm, irregular, bluish white translucent mass with a smooth and glistening surface resembling the ovary in shape. It measured 9 by 6 by 3 cm., and weighed 100 gm. On sectioning the mass the knife met with considerable resistance. The cut surface consisted of firm, bluish white fibers enclosing small areas of pink tissue, and was everywhere translucent and very firm. Microscopically the tumor was composed of ovarian type of stroma, rich in cells and fibrous tissue with nests of epithelial cells, characteristic of the Brenner tumor. The pseudomucinous cystoma type of Brenner's tumor occurred in the left ovary, which was converted into a round fluctuating mass with an intact outer membrane whose

surface was smooth and glistening. The mass measured 20 cm. in diameter and weighed 3000 gm. On sectioning the mass it was found to consist of one large cavity with many smaller cysts projecting from the inner surface of its thick, bluish white, translucent fibrous wall. The large and smaller cysts were filled with a mucoid material and lined by a pink membrane, mostly smooth but in a few places mass-like. Microscopically the cyst was lined by pseudomucin secreting columnar epithelium. In the stroma of the wall were epithelial nests and strands of the Brenner type. Both tumors were considered benign. This was apparently the first recorded bilateral case.

(Dr. Alfred Plaut, New York City.) As to the rarity of the Brenner tumor, probably the same rule applies as to many other rare conditions: once it has been demonstrated convincingly it will be found more frequently. In fact, the number of case reports has increased since Robert Meyer's publication. Robert Meyer has observed 4 cases in 20 years in his enormous material in Berlin. In Budapest 5 cases have been seen among 1100 ovarian tumors, and we have seen eight Brenner tumors within 9 years in New York City. When I first saw these tumors I had a disagreement with the tumor authorities in New York because they insisted that these nodules were malignant while I considered them benign. Today we know they are benign. Whether there is a potential malignancy I do not know, but so far no case has turned malignant. There is a statement in the literature that one Brenner tumor in Kermauner's collection in Vienna showed something like malignancy. But this case represents only a metastasis of another carcinoma into that part of an ovary which incidentally is surrounding a Brenner tumor. When I saw a low power photomicrograph of this case in the Handbook of Gynecology I wrote a letter to the Kermauner Clinic in Vienna asking if this were not perhaps a Brenner tumor. Dr. Schiller was kind enough to look the case up and he found it to be a Brenner tumor. At the same time, 2 other cases from Kermauner's collection, which had been put on record as metastatic carcinoma in the ovary, also have been classified as Brenner tumors. Bilateral Brenner tumors must be very rare. Did you find solid tumors in both ovaries?

(Dr. Schmeisser.) No, solid on one and the other side was cystic, and Brenner cells were found in the cyst.

(Dr. Plaut.) Schiffmann has reported a Brenner tumor in one ovary, with Brenner tumor found microscopically in the other ovary. The Brenner tumor in an unusually large percentage of cases is found together with other ovarian tumors, also with rare ones. It has been found together not only with carcinoma of the ovary, but also with granulosa cell tumors and with ovarian struma. Brenner was not the first one to describe this tumor. Eight years before him, Orthmann described the tumor very well and gave good illustrations. Neither he nor Brenner has interpreted the tumors correctly. It was Robert Meyer in Berlin who gave the first correct interpretation.

I am calling the tumor "fibroepithelioma mucinosum benignum," thinking that this name describes the most important features of the Brenner tumor.

Within the last 2 years I have found only one additional Brenner tumor in the material of the Beth Israel Hospital.

PAPILLARY CYSTADENOMA LYMPHOMATOSUM OF THE PAROTID GLAND (ONKOCYTOMA). David A. Wood, San Francisco, Calif.

Abstract. Cystadenomas of the salivary glands comprise an unusually typical but rare group of tumors. To date 31 authenticated cases confirmed by histo-

logical examination have appeared in the literature. Extensive speculation as to their obscure origin has led to much confusion in nomenclature. For example, we find such terms as onkocytoma, adenolymphoma, branchiogenic adenoma, "orbital inclusion" cystadenomas, and papillary cystadenoma lymphomatosum.

Clinically and pathologically these tumors are slowly growing, benign, occur chiefly in males beyond 40 years of age, and show a characteristic structure except for cystic, tubular, and papillary variations. Papillary cystadenoma lymphomatosum is a descriptive name and refers to the intimate admixture of epithelial and lymphadenoid components which are characteristic. Their lining walls are characteristic in that they are composed of a double layer of peculiar, tall columnar epithelium, the cells of which are swollen, granular, possess distally placed nuclei, and show no cilia. Interstitial secretion capillaries are fairly numerous. The lymphadenoid stroma contains what appears to be definite germinal centers.

Three additional cases are reported, all occurring in males, 37, 48 and 71 years of age. The tumors were all approximately the same size, averaging 5 by 4 by 3.5 cm. They were similar in structure except for papillary and cystic variations. The three tumors were sharply encapsulated and circumscribed. Two were found in the right parotid gland, one in the left.

In support of the thesis that papillary cystadenoma lymphomatosum is a true salivary tumor, another case is presented in which a parotid gland from a cadaver showed a cystic dilatation of an excretory duct. The duct was partially lined by a double layer of columnar epithelial cells supported upon a lymphadenoid stroma presenting a picture strikingly similar to that seen in the 3 cases reported. These peculiar epithelial cells known as "onkocytes" can be found in the salivary glands of progressively aging individuals beyond the age of 20 years. Their occurrence is always associated with the development of a lymphadenoid stroma. The similarity between "onkocytes" and the cells characteristically found in the tumors under discussion is so striking as to suggest the possible adoption of the name "onkocytoma."

THE EFFECT OF PITUITARECTOMY ON THE NATURAL RESISTANCE OF ADULT ALBINO RATS TO HISTAMINE POISONING. David Perla and (by invitation) S. H. Rosen, New York City.

Abstract. The natural resistance to histamine was depressed in completely pituitarectomized rats 1 to 10 weeks after operation. The MLD was one-fifth to one-third that for normal rats.

This decrease in resistance was associated with hemorrhage into or atrophy of the inner zones of the cortex of the adrenals.

Rats in which the posterior lobe and most of the anterior lobe were removed showed a similar drop in resistance. In these instances atrophic changes in the suprarenal cortex occurred. Where a large fragment of anterior lobe remained there was no depression of resistance to histamine and the suprarenal glands were normal.

The repeated injections of large amounts of suprarenal cortical hormone raised the natural resistance of totally pituitarectomized adult rats to histamine poisoning. In some instances the resistance was raised almost to the level of normal rats.

The drop in natural resistance to histamine following pituitarectomy in the rat is probably secondary to the atrophic changes of the suprarenal cortex induced by the withdrawal of the adrenotropic hormone of the anterior lobe.

Discussion

(Dr. David Seecof, Montreal.) I should like to ask how these quantitative determinations were made. Was the lethal dose of the histamine determined by noting whether a rat succumbed to 100 mg., or to 1000 mg., or was the dose repeated daily, or every few hours; in other words, how was the exact quantity determined for the lethal dose of histamine?

(Dr. Perla, closing.) The lethal dose was determined only by groups of rats. A series of rats was taken in which the hypophysis was completely removed; some were given one dose, and others a larger dose, and in that way the range was established. In some instances a rat was used more than once, but the interval between was generally several weeks. The minimum lethal dose could not be determined with a drug such as histamine on an individual animal, but simply the range of a number of animals receiving the same operative procedure.

PITUITARY BASOPHILISM. H. M. Zimmerman, New Haven, Conn.

Abstract. The case is described of a white male, aged 44 years, who presented the clinical features of Cushing's syndrome of pituitary basophilism. The patient came to autopsy as the result of a ruptured dissecting aortic aneurysm and cervical cellulitis. Postmortem examination revealed an inactive basophilic adenoma of the anterior pituitary, cortical hyperplasia and adenomas of the adrenal glands, an adenoma of one of the parathyroid bodies, and diffuse skeletal demineralization.

Discussion

(Dr. William Boyd, Winnipeg.) Was there any calcification in the kidneys? Could the adenoma be recognized with the naked eye? Was it clearly demarcated by a capsule from the surrounding tissue?

(Dr. H. Edward MacMahon, Boston.) I did not understand what kind of an aneurysm was present. I should be interested in knowing if this were a dissecting aneurysm because of the frequency of this lesion in malignant hypertension and of the association of the latter with functioning basophilic adenomas of the adenohypophysis.

(Dr. Zimmerman, closing.) It was a dissecting aneurysm.

In answer to Dr. Boyd's questions — there was no calcification in the kidney. The arteries and arterioles in the kidney appeared normal.

The tumor could not be seen with the naked eye, and we escaped losing it by not stripping the dural envelope of the pituitary. Frequently it has been our custom to remove the dural envelope to facilitate sectioning, and it is possible to tear away the adenomas as they occur beneath the dura, and frequently, as in this case, invade the dural envelope. The tumor was sharply demarcated. It was outlined by a thin, connective tissue capsule, and it had compressed the surrounding pituitary cells so that it did represent an actual adenoma.

A HISTOPATHOLOGICAL STUDY OF ONE HUNDRED HYPOPHYSES. E. M. Butt (by invitation) and Roy M. Van Wart, Los Angeles, Calif.

Abstract. In a study of 126 hypophyses from unselected cases of all ages, an attempt was made to correlate the histological findings with clinical and pathological changes. The hypophyses were serially sectioned on a horizontal plane at 7 microns, every tenth section being mounted and stained with hematoxylin and eosin. From 50 to 90 sections were examined for each hypophysis.

On account of the brief period of time allotted, the presentation was limited to the subject of infiltration of the pars nervosa by epithelial elements, presumably arising from the pars intermedia. Epithelial infiltration of the posterior lobe was found in 83.6 per cent of all cases. The greatest incidence was noted in the age group from 61 to 70. However, good examples of cellular infiltration of the pars nervosa were found in individuals under 30 years of age. No correlation was established between hypertensive states, atherosclerosis or eclampsia and posterior lobe basophilia.

Discussion

(Dr. Alfred Plaut, New York City.) We have in the last 2 years undertaken a similar study comprising about 80 hypophyses. We again are unable to find any relation between the amount of epithelial infiltration of the posterior lobe and the blood pressure. In the course of other hypophysis studies we have (many years ago) attempted to find a correlation between this epithelial invasion and any other condition. We found none. I should like to ask Dr. Butt how many different levels of these hypophyses were examined.

(Dr. Butt.) Serial sections were cut at 7 microns. Every tenth section was examined.

(Dr. Plaut.) Since this was so, I am astonished that you found such a high percentage of hypophyses without infiltration, because if one examines a large number of sections, one finds infiltration practically always in adults, and in children also.

(Dr. David Seecof, Montreal.) I might mention casually some observations on about 2000 pituitaries not sectioned serially, but on taking one section through the middle of the gland. The infiltration of the posterior lobe was found very often and no correlation with any clinical or pathological condition could be found.

(Dr. William Boyd, Winnipeg.) My own experience agrees entirely with the speakers.

(Dr. Butt.) In closing I might say that some 84 per cent had epithelial infiltration of basophilic type, and only 17 per cent were without it. I assume if we had studied every fifth section we would have found a higher percentage of infiltration in all ages.

MORPHOLOGICAL EVIDENCE OF THE EFFECT OF IODINE AND DESICCATED THYROID ON THE ANTERIOR PITUITARY. David Marine and (by invitation) S. H. Rosen and C. Spark, New York City.

Abstract. It has been known for a century that the anterior pituitary is markedly enlarged in individuals with endemic goiter and for more than 50 years that removal of the thyroid is followed by hypertrophy of the anterior pituitary and disappearance of the acidophilic granules. Our experiments on rabbits have shown that iodine administered to rabbits with hyperplastic goiter and hypertrophic anterior pituitary causes a restoration of the acidophilic granules and a great shrinkage of the volume of the gland amounting to a return to normal size and staining reaction.

Iodine administered to thyroidectomized rabbits neither prevents the occurrence of hypertrophy nor restores the acidophilic granules. Desiccated thyroid or thyroxine will prevent the hypertrophy and restore the acidophilic granules and reduce the volume of the gland to normal if hypertrophy has occurred.

These studies indicate that the thyroid secretion exerts as remarkable a controlling influence on the anterior pituitary as the anterior pituitary hormone (thyrotropic) does on the thyroid.

Discussion

(Dr. Isolde T. Zeckwer, Philadelphia.) In some experiments which we have been carrying out the pituitary of thyroidectomized rats was tested for its thyrotropic hormone potency by injection into guinea pigs. The thyroidectomized rat's pituitary at a stage when it is almost devoid of acidophiles has a very high thyrotropic hormone content, greater than that of normal rat pituitary controls. I believe this is direct evidence that the acidophile can be excluded as the producer of the thyrotropic hormone.

(Dr. Marine, closing.) We have not tried rats. Dr. Rosen has tried several such experiments on the rabbit pituitary and we could not find any evidence that the thyrotropic factor was increased in thyroidectomized rabbits, in spite of the fact that after thyroidectomy there is other evidence to believe that the thyrotropic hormone is formed in much greater amounts. Our opinion is that it is not being stored. Others have reported similar findings for the dog and rat. Aaron has shown an increase in the thyrotropic factor in the peripheral blood of thyroidectomized animals and several laboratories have observed the increase in acidophilic granules and in thyrotropic potency of pituitaries following iodine administration to animals with intact thyroids.

READ BY TITLE

- MASSIVE LEFT AURICLE. L. F. Bishop, Jr., and (by invitation) Andrew Babey, New York City.
- A STUDY OF BACTERIAL CAPSULES WITH SPECIAL REFERENCE TO THE MODIFIED USE OF INDIA INK. E. M. Butt (by invitation), Los Angeles, Calif.
- THE EFFECT OF X-RAY ON ENCEPHALITIS LETHARGICA. S. A. Goldberg, M. Brodie and (by invitation) P. Stanley, Newark, N. J.
- EXPERIMENTAL SUBACUTE TULAREMIA IN RABBITS. R. D. Lillie and (by invitation) E. Francis, Washington, D. C.
- FURTHER STUDIES ON THE MEASUREMENTS OF THE MACRONUCLEOLUS OF CANCER. William Carpenter MacCarty, Rochester, Minn.
- BACTERIOPHAGE SERVICE TO ONE HUNDRED STAPHYLOCOCCUS SEPTICEMIAS. Ward J. MacNeal and (by invitation) Frances C. Frisbee, New York City.
- COMPLEMENT FIXATION AS A DIAGNOSTIC TEST IN BLASTOMYCOSIS. Donald S. Martin (by invitation), Durham, N. C.
- ABSORPTION OF INHALED PROTEINS FROM THE UPPER RESPIRATORY PASSAGES INTO THE BLOOD STREAM. Bret Ratner, New York City.
- FAMILIAL BONE ABNORMALITIES IN THE RABBIT. Paul D. Rosahn, New York City.
- SUBMAXILLARY GLAND DISEASE OF THE MOUSE. Juanita Thompson, New York City.

